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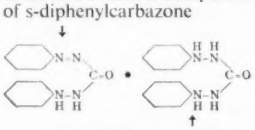

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LEPTOSPIROSIS†

I. INTRODUCTION

Leptospirosis is a disease of growing world-wide importance. During the past three decades the number of human infections recognized in this country has increased at an ever mounting rate, so that reports of 20 to 80 cases from a single city have become commonplace.[1,2] This trend, which promises to continue, can probably be related to the growing alertness of clinicians to the possibility of infection, the evolution of a broader concept of the clinical picture to be expected, expanding knowledge of the distribution of leptospirae in animals, and, perhaps most important of all, the wider availability of diagnostic laboratory facilities.

There are a large number of antigenically distinct strains of pathogenic leptospirae,[3,4] but only six have thus far been implicated as sources of human infection in the United States: *L. icterohaemorrhagiae,*[5] *L. canicola,*[6] *L. pomona,*[7] *L. grippotyphosa,*[8] *L. autumnalis,*[9,10] and *L. bataviae.*[11] In addition, several other pathogenic strains have been recovered from rodents,[12] but as yet have not been found in man.

Leptospiral strains may vary not only in antigenic structure but also in epidemiological and clinical behavior. Nevertheless, the infections they produce in man invariably follow direct or indirect contact with animals that serve as their natural reservoirs, and are all characterized by early leptospiremia and an acute febrile reaction of abrupt onset and relatively brief duration. In addition, many strains invade specialized tissues producing lesions in the liver, kidneys, meninges, and eyes, which not only prolong the disease but also give rise to many of its most distinctive clinical features. Thus, classical Weil's disease, an infection due to *L. icterohaemorrhagiae,* is usually accompanied by jaundice and signs of renal injury, while swineherd's disease, an infection due to *L. pomona,* is usually characterized by meningitis. Although the lesions produced by each of the leptospiral strains tend to fall into a distinctive pattern, they are by no means constant, and most strains are potentially capable of producing the same lesions. Thus, any given strain may fail to produce localizing signs, or may give rise to

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* Associate Professor of Medicine.

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hepatitis, nephritis, or meningitis, either alone or in combination. Of interest in this connection is the fact that the same strain may produce different clinical manifestations in various parts of the world. Recent experience in this country with *L. grippotyphosa, L. autumnalis,* and *L. pomona* bears this out.

Nomenclature presents something of a problem in any discussion of leptospirosis. The term Weil's disease, which usually implies an infection with *L. icterohaemorrhagiae,* is sometimes also applied to any leptospirosis accompanied by jaundice, or, even more loosely, to leptospiral infections in general. Since jaundice is an inconstant finding, even in Weil's disease, it would appear to be an inadequate basis for classification. Moreover, while the infections produced by many leptospiral strains closely resemble Weil's disease, their epidemiological features are often very different. For the sake of clarity, therefore, the term Weil's disease should be restricted to infections with *L. icterohaemorrhagiae.* On the whole, however, the term leptospirosis, qualified wherever possible by the specific strain, is to be preferred, since it is more informative and less likely to be confusing. In some cases, localizing signs in the meninges, kidney, or eye are such a striking feature of the disease that use of the terms leptospiral meningitis, nephritis, or uveitis would appear to be justified, even if only to underline the unusual etiology of a lesion commonly due to other causes.

A number of other terms are also used to describe leptospiral infections in various parts of the world.[3, 4] These are based either on the geographical distribution of the disease or on one of its clinical or epidemiological features. For the most part these came into use before the etiology of the disease was known, so that it is not surprising that infections with the same strain of leptospira are often known by a multiplicity of names. Although confusing, there may be some justification in retaining these names, since the clinical and epidemiological behavior of some strains appears to vary in different parts of the world. Some of the names used in connection with the leptospiral strains recovered in this country are of interest: (1) *L. canicola*—canicola fever, (2) *L. grippotyphosa*—mud fever, water fever, harvest fever, or summer influenza (Europe), (3) *L. pomona*—swineherd's disease (Europe), Pomona or 7-day fever (Australia), (4) *L. autumnalis* —autumnal fever, Hasami fever, or Akiyami (Japan), Fort Bragg or pretibial fever (U.S.A.), (5) *L. bataviae*—Indonesian Weil's disease.

II. ETIOLOGY

1. *Classification of leptospirae.* Leptospirae are classified on the basis of their antigenic structure as revealed by cross agglutination-lysis and cross agglutination-absorption tests with specific antisera. Recent evidence[10]

indicates that there are at least 36 antigenically distinct strains or serotypes. Some of these exhibit variations in the complexity of their antigenic structure and are accordingly further subdivided into complete and incomplete biotypes. The individual strains show striking differences in virulence, geographical distribution, natural hosts, and the clinical manifestations they produce. However, as previously indicated, the latter are not sufficiently characteristic or constant to permit distinction between the various types of leptospirosis.

2. *Characteristics of leptospirae.* All strains of leptospirae exhibit identical morphological features, even under the electron microscope.[18] Characteristically, they are long slender structures, measuring 6 to 9 microns in length and 0.25 micron in breadth, with sharp, pointed ends that are hooked either in the same or opposite directions. Tightly coiled, minute spirals running their length give them a beaded appearance under dark-field illumination.[14] Recent electron microscope studies[13, 15] reveal that the body of the organism is entwined by a fine spiral filament, and it has been suggested[18] that this structure may be contractile and responsible for the characteristic undulating, twisting, and propulsive movements of the leptospira. Minute forms of the organism are thought to occur, since Berkfeld filtrates containing no visible leptospirae are sometimes capable of infecting guinea-pigs.[14] In liquid media leptospirae are best demonstrated by means of dark-field illumination. Although it is possible to stain them in dried thick films by the Giemsa technique, silver stains are required for their demonstration in tissues.

Leptospirae may be cultivated at a temperature of 25 to 30 degrees centigrade in a variety of media containing guinea-pig kidney, whole blood, or serum, and may be grown in the chorioallantoic membrane of the chick embryo. They may also be propagated in susceptible animals. Young guinea-pigs are used most widely, but since they are relatively resistant to such strains as *L. canicola*[16] and *L. grippotyphosa*,[5] hamsters and white mice are often employed. A wide variety of other rodents and domestic animals have been shown to be susceptible to experimental infections. Characteristically, leptospiral infections are followed by the appearance of specific serum agglutinins, lysins, and complement-fixing antibodies. Although cross-agglutination and lysis with other strains of leptospirae often occur, the titer against the homologous strain is usually significantly higher. Agglutination tests are reasonably reliable, therefore, in distinguishing between various leptospiral infections and in identifying isolated organisms. However, for the most accurate identification, cross absorption and neutralization tests are required. The complement-fixing antibodies that appear after

infection, while as diagnostic of leptospirosis as the agglutinins, are far less type specific.[17]

3. *American strains of leptospirae.* Most human leptospiral infections in this country are due to *L. icterohaemorrhagiae,* the most virulent of the six strains identified. It produces not only classical Weil's disease with jaundice, but also many anicteric infections, some of which are accompanied by meningitis or nephritis.

L. canicola is a less common case of leptospirosis and usually produces a much milder disease. In other respects, however, the infection is indistinguishable from that produced by *L.icterohaemorrhagiae.*[6, 14]

Only a small number of *L. pomona* infections have been reported thus far.[7, 18–21] However, the disease is so prevalent in cattle and swine[22–25] that it is quite possible that many human cases have been overlooked, particularly since the infection is rarely accompanied by jaundice and is, thus, likely to be confused with other diseases. Meningitis has been the outstanding feature in many American cases,[18, 20, 21] just as in swineherd's disease, the European version of *L. pomona* infection.[26] Serological evidence suggests that *L. bovis* in cattle and *L. pomona* are identical.[22]

Recently it has been shown that *L. autumnalis* is the agent responsible for Fort Bragg or pretibial fever, an anicteric infection characterized by fever, splenomegaly, and pretibial rash.[9, 10] The disease was first recognized in Georgia[27] and later among soldiers stationed at Fort Bragg, North Carolina in 1942.[28] It recurred in the latter area during the following two years, but has not been seen again. A filtrable agent, thought to be a virus, was recovered from the blood of one patient and was successfully passed through guinea-pigs, hamsters, and embryonated eggs.[29] Curiously, the true nature of the infection was not discovered until eight years following the last outbreak, when leptospirae resembling *L. autumnalis* were recovered from the blood of hamsters after the 365th serial passage through guinea-pigs, embryonated eggs, and hamsters.[9] Recently the antigenic structure of this organism has been shown to be more complex than that of *L. autumnalis,* Akayami A, the agent responsible for autumnal fever in Japan, and, accordingly, the special designation, *L. autumnalis,* Fort Bragg, has been adopted.[10]

Only one instance of an *L. grippotyphosa* infection has been reported in this country.[8] It occurred in a Texas farmer and was accompanied by jaundice. In Eastern Europe the same organism produces a mild nonicteric febrile illness known as mud fever or summer influenza.[4]

Recently, serological evidence of an *L. bataviae* infection was obtained in a Louisiana trapper.[11] No details of the clinical features in this case are

available. However, in Java the same strain is known to produce a moderately severe illness with jaundice known as Indonesian Weil's disease.[4]

III. EPIDEMIOLOGY

1. *Animal hosts.* Leptospiral infections in man are almost invariably the consequence of close contact with animals or their urine. The species that harbor these organisms do not necessarily suffer any ill effects, although some develop an illness which closely resembles that seen in man.

As the principal host of *L. icterohaemorrhagiae,* the rat is responsible for most cases of Weil's disease. Although the dog is also capable of transmitting the disease, it does so only infrequently.[4] *L. icterohaemorrhagiae* has also been recovered from a variety of other animals, including field mice, cats, pigs, and horses,[5] but these do not appear to be responsible for infections in man. The sewer rat (*Rattus norvegicus*) is the principal vector of the disease, but house rats (*Rattus rattus* and *Rattus rattus alexandrinus*) have also been implicated.[4] A high proportion of these animals have been found infected in all parts of the world where surveys have been carried out.[30, 31] In the United States the incidence of infection in the rat population of urban areas varies from 25 to 35 per cent.[32, 33] Leptospirae, which appear to produce no ill effects in the rat, are excreted in large numbers in the urine, and can be demonstrated in the kidney, but not in the blood, liver, or other tissues.[31] The infection is transmitted from rat to rat by contact with infected urine or urine-contaminated food and water, and possibly through sexual intercourse.[34]

Dogs constitute the only natural reservoir of *L. canicola.* The rat is peculiarly resistant, but a number of other animals can be infected experimentally.[8] As previously mentioned, dogs may also harbor *L. icterohaemorrhagiae,* but do so far less frequently than *L. canicola.*[30] In both instances the animal may suffer a highly lethal acute illness manifested by jaundice, hemorrhagic gastroenteritis, or nephritis.[8] However, serological studies in healthy dogs have revealed an unusually high incidence of positive agglutination reactions,[16, 33] suggesting that mild and inapparent infections must be relatively common. Not infrequently chronic nephritis is a sequel of acute leptospiral infection in dogs and is accompanied by prolonged leptospiruria. Transmission of the infection from dog to dog is the result of contact with infected urine or urine-contaminated food and water. It may also be acquired through sexual intercourse.[4]

L. pomona has been recovered from cattle and swine in many parts of this country,[22–25] and serological evidence[18] suggests that it also occurs in horses and mules but not in rats. The infection is often asymptomatic in

pigs, but may cause abortion[35] or give rise to mild constitutional symptoms.[4] In cattle the disease tends to be more severe and is occasionally fatal, being accompanied by fever, jaundice, and hemoglobinuria.[29] However, asymptomatic infections and uncomplicated abortion also occur. Little is known about the epidemiology of leptospirosis in horses, but recent serological evidence[35] points to *L. pomona* as the etiological agent in recurrent iridocyclitis, a disease that affects this species.

The reservoir of *L. autumnalis,* Fort Bragg, is unknown. However, the closely related strain found in Japan, *L. autumnalis,* Akayami A, is carried by field mice.[4]

In Eastern Europe the vole, a type of mouse, is the natural host of *L. grippotyphosa.*[4] Whether this species was responsible for the only human infection reported in this country[8] is not known. Similarly, the source of the single *L. bataviae* infection observed in Louisiana[11] was not determined. However, in Indonesia, where the disease is common, the sewer rat appears to be the principal carrier.[4]

2. *Transmission of leptospirosis to man.* With few exceptions human infections with *L. icterohaemorrhagiae* can be traced to direct or indirect contact with contaminated rat urine. Occasionally, the disease is also acquired as the result of rat bite,[21] accidental contact with live cultures in the laboratory[30] or handling of infected rat tissues. Man-to-man and intrauterine fetal infections are said to occur rarely,[5] and at least one instance of probable venereal transmission has been reported.[30] The abraded skin and the intact mucous membranes, especially of the conjunctival sac, nose, mouth, and throat, serve as the usual portals of entry. There is considerable doubt that leptospirae can penetrate the intact skin,[30] and it is unlikely that they gain entry through the stomach and upper intestinal tract, since they are readily destroyed in an acid medium.

Although Weil's disease may be acquired in any rat-infected environment, certain occupations provide peculiarly favorable conditions for infection, so much so, indeed, that some states now recognize leptospirosis as a compensable occupational disease.[34] In this country fish cutters and cleaners, butchers, miners, rat-catchers, sewer workers, plumbers, tunnel diggers, garbage handlers, and dock workers are particularly subject to the disease.[2, 5, 34] As might be expected, infected dogs constitute a special hazard for veterinarians and other dog handlers.[8] In other parts of the world, especially in tropical and subtropical areas, the disease is particularly likely to affect sugar-cane cutters[30] and rice-field workers who are obliged to work in contaminated irrigated fields. During warfare soldiers not infrequently become infected in poorly drained trenches. In view of the occupations that

predispose to Weil's disease it is not surprising that 90 per cent of the cases occur in adult males, and that only rarely are children affected.[2]

Swimming and accidental immersion in rat-contaminated stagnant bodies of water constitute another important mode of acquiring Weil's disease. Although swimming is thought to be the less hazardous of the two, outbreaks have been reported amongst swimmers both in this country[37] and abroad.[2, 28, 30]

Certain environmental factors favor the spread of leptospirae and account, in part at least, for many of the epidemiological features of Weil's disease. These include dampness, a temperature of 22 to 25 degrees centigrade, and a neutral or slightly alkaline medium in which leptospirae may survive for as long as three weeks.[4] Accordingly, the summer months provide the most favorable conditions for transmission of the disease in this country.[5] However, the expected summer peak has not been observed in all series.[2]

L. canicola infections usually follow close contact with dogs that are shedding leptospirae in their urine.[6] Since the organism survives in neutral water, as in the case of *L. icterohaemorrhagiae,* infection may also occasionally be acquired by swimming in stagnant contaminated water. Considering the high incidence of leptospirosis in dogs and the opportunities for close contact with man, human infections are surprisingly few in number. Of probable importance in this connection is the fact that the dog is only a temporary shedder of leptospirae, seldom contaminates its environment to the same extent as the rat, and has an acid urine which does not permit long survival of organisms.[16]

L. pomona infections in this country have followed close contact with pigs[19] and cattle,[20] handling of raw beef and pork,[7, 21] and swimming in water presumably contaminated by infected hogs.[18] Evidently, pigs are a more common source of infection than cattle, although further experience with the disease may not bear this out. As in the case of *L. icterohaemorrhagiae,* the organism gains entrance to the body through the broken skin or intact mucous membranes. However, in contrast to the other leptospiroses, in which direct or indirect contact with infected urine is the principal mode of infection, the handling of infected tissues would appear to be an additional hazard peculiar to this disease.

As previously indicated, the epidemiological features of the few *L. autumnalis,* Fort Bragg, *L. grippotyphosa,* and *L. bataviae* infections reported in this country have not been elucidated. However, experience with these organisms in other parts of the world suggest that contact with contaminated rodent urine will prove to be the cause of infection.

IV. PATHOGENESIS

1. *General features.* Following an incubation period of 7 to 13 days (extremes 4 to 19 days),[30] and coincidental with the onset of symptoms, *L. icterohaemorrhagiae* which have gained access to the body make their appearance in the blood in large numbers, and are soon disseminated to most, if not all, of the tissues.[14] By the end of a week, however, the leptospirae have usually vanished from the blood and can seldom be found in any of the tissues except the kidney[14, 40, 41] and occasionally the liver.[14] Rarely, a few degenerate organisms may be demonstrable in other tissues as late as the sixteenth day of the disease.[14] It is generally assumed that the disappearance of organisms from the blood and tissues is related to the development of immune bodies,[14] although diagnostically significant titers are seldom demonstrable until several days to a week later. The period of leptospiremia is characterized by severe constitutional symptoms of toxic origin.

Clinical manifestations related to the development of lesions in the liver, kidneys, and other special tissues do not become evident until the end of the first week, or even later, at which time leptospiremia is diminished or has actually ceased. Since most fatalities occur in the second or third week of the disease,[8, 14] little is known about the early lesions in man. However, the autopsy findings in several cases that have died on the fifth or sixth day[40, 41, 43] suggest that tissue injury may occur during the stage of leptospiremia and before localizing signs become evident. Of interest in this connection is the observation that in experimental animals leptospirae may be demonstrable in the liver before they appear in the blood.[43]

The liver, kidneys, muscles, blood vessels, and meninges are the tissues most frequently involved in *L. icterohaemorrhagiae* infections, but others may be affected in individual cases.

In general the behavior of the other strains of leptospirae found in this country is very similar to that of *L. icterohaemorrhagiae.* However, the infections they produce in man tend to be less severe, and are less likely to be accompanied by signs of hepatic and renal involvement. These will be discussed more fully in Section V. The tissue changes found in fatal cases of *L. canicola* infection resemble those of Weil's disease.[44] Their nature in the other leptospiroses found in this country is not known, since no fatalities have been reported.

2. *Pathologic anatomy*

(a) *Liver.* One of the striking features of Weil's disease is the remarkable discrepancy between the severity of the jaundice and the extent of the hepatic lesions found at autopsy. The histological changes usually observed include (1) dissociation and disorganization of the centrilobular paren-

chymal cells, (2) active hepatocellular regeneration, as evidenced by the presence of numerous binucleate cells and mitoses, (3) minimal degenerative changes in the cytoplasm and nuclei of the parenchymal cells, (4) bile stasis, especially in the centrilobular canaliculi, and (5) a predominantly mononuclear infiltration of the portal zones.[41, 42, 45] In the most severe cases, small areas of focal necrosis occur and, rarely, massive hepatic necrosis may supervene.[46] However, it should be emphasized that necrosis is distinctly unusual, and that the liver may actually show no changes whatever even in the presence of deep jaundice.[42] It is difficult to account for the unusual regenerative activity of the parenchyma which is such a striking feature in this disease. It would seem reasonable to suppose that it represents a response to antecedent cellular necrosis. However, serial studies of the liver in experimentally infected animals[48] do not support this interpretation, mitotic activity being demonstrable in the absence of necrosis, just as in man. Leptospirae can be demonstrated in the liver in only half the cases that come to autopsy. They are usually found overlying the parenchymal cells but do not appear to penetrate them.[43]

The jaundice of Weil's disease is almost certainly due to hepatocellular injury, although increased hemolysis may be a contributory factor. Since jaundice is occasionally seen in the absence of histologically demonstrable cellular changes,[42] it must be assumed that apparently intact parenchyma may suffer a functional derangement in bilirubin excretion. There is no evidence that extrahepatic biliary obstruction plays any rôle in the jaundice of Weil's disease, and the bile thrombi found in the canaliculi are generally considered the consequence rather than the cause of cellular injury and bile stasis. The presence of phagocytized erythrocytes in the liver, spleen, and sites of hemorrhage[43] is consistent with the impression that the rate of hemolysis is often increased. Although hemolysis undoubtedly contributes to the jaundice under these circumstances, it is obviously not its sole cause since the degree of anemia produced is seldom of sufficient severity to account for the depth of the accompanying jaundice. Moreover, the clinical and chemical features of the jaundice are not those of a predominantly hemolytic process.

Since non-icteric leptospirosis is rarely fatal, it is not known whether it is associated with any morphological changes in the liver. However, alterations in hepatic function have been observed in some,[9] but not all cases.[47]

(b) *Kidneys.* In general the renal lesions in Weil's disease[5, 42, 45, 48] are more impressive than those in the liver, and leptospirae are more frequently demonstrable. When present, the latter are found both in the tubules and interstitial tissue.[5, 40] The kidneys are usually enlarged and moderately bile

stained. Microscopically, they show: (1) degeneration and necrosis of the convoluted tubules, (2) a diffuse infiltration of the interstitial tissue by lymphocytes, monocytes, and occasional polymorphonuclear and eosinophilic leukocytes, (3) numerous granular casts and cellular debris in the distal convoluted and collecting tubules, and (4) small hemorrhages into the capsule, interstitial tissue and tubules. The glomeruli are usually intact, but occasionally show changes resembling those of acute glomerular nephritis.[5]

The lesions in the kidney are almost certainly due to the invasion of leptospirae and are unrelated to the accompanying hepatic failure. As might be expected, they frequently produce a type of renal insufficiency that closely resembles that seen in lower nephron nephrosis due to other causes.

(c) *Muscles.* Highly characteristic lesions can usually be demonstrated in the skeletal muscles in *L. icterohaemorrhagiae* infections, and their number can be correlated with the severity of the disease.[49] It is quite possible that they are responsible for the myalgia which is such a striking clinical feature. Early in the disease individual fibers show localized areas of vacuolization with loss of striations and multiplication of adjacent sarcolemma nuclei. Later, sarcolemma cells invade these necrotic foci and remove any remaining debris. In mild cases, new myofibrils appear within these cells, while in more severe cases the foci are infiltrated with lymphocytes and plasma cells and undergo fibrosis. Leptospirae have not been demonstrated in any of these lesions, although they have not been studied before the eighth day of the disease. The absence of hemorrhage and vascular lesions serves to distinguish them from Zenker's degeneration and the lesions seen in rickettsial disease.

(d) *Blood vessels.* The serosal surfaces, mucous membranes, and many of the viscera frequently exhibit petechial hemorrhages. In more severe cases, there may be purpura of the skin and gross bleeding into the gastrointestinal tract, pulmonary tree, or urinary tract. Since there is no reduction in the platelet count or prolongation in the bleeding or coagulation time, it is generally believed that the hemorrhages are due to capillary injury.[5] The prothrombin concentration is usually only slightly to moderately decreased.[50] However, it occasionally falls to very low levels in severely jaundiced cases,[84] and may, thus, contribute to the hemorrhagic tendency.

(e) *Meninges.* The meninges are often the site of a low-grade lymphocytic and plasmocytic inflammatory reaction.[5] This is usually accompanied by pleocytosis of the cerebrospinal fluid. Degenerative changes are seen in the neurones, but there is no inflammatory reaction in the substance of the brain or spinal cord.

(f) *Heart.* A variety of lesions may occur in the heart. These include: (1) hemorrhages into the epicardium and endocardium, (2) round-cell infiltration and degenerative changes in the myocardium resembling those seen in skeletal muscles,[34] and (3) rarely, acute vegetative endocarditis.[8]

V. CLINICAL MANIFESTATIONS[4, 5, 49, 50]

As previously indicated, the clinical picture in leptospirosis varies, depending not only on the virulence of the particular strain involved, but also on its predilection for certain tissues.

1. *Weil's disease.* In *L. icterohaemorrhagiae* infections the clinical manifestations emerge in two phases. Early in the disease there are severe constitutional symptoms related to the presence of leptospirae in the blood. Later, localizing signs appear pointing to invasion of specialized tissues, especially in the liver, kidneys, and meninges. The two phases overlap to some extent, and in some cases localizing signs fail to develop. In the classical form of the disease the latter half of the clinical course is usually dominated by signs of hepatic and renal involvement. However, these may be lacking, and the infection may present as a simple acute febrile episode or as an acute aseptic meningitis.[51, 59] This is particularly likely to occur in mild *L. icterohaemorrhagiae* infections and is characteristic of many of the infections induced by other less virulent strains.

Characteristically, the onset of symptoms in Weil's disease is sudden, with fever, headache, and severe prostration. Muscle pain, especially in the calves and lumbar region, is usually a prominent feature and may be accompanied by local tenderness. Chilly sensations are common, and occasionally a shaking chill ushers in the disease. Marked conjunctival injection is usually present and is a helpful diagnostic sign. As the disease progresses, hemorrhagic herpes labialis, gastrointestinal symptoms, nuchal rigidity, and cough often appear. Morbilliform and scarlatiniform rashes are seen in approximately 10 per cent of cases. At this stage, leptospirae can be demonstrated in the blood, but physical signs, other than those of a severe infection, may be completely lacking. Leukocytosis is the rule, and the cerebrospinal fluid often contains an increased number of cells.

Toward the end of the first week, as the leptospirae are disappearing from the blood, the temperature falls, but the patient's general condition usually worsens. Although muscular aching and many of the other constitutional symptoms diminish, prostration, anorexia, and gastrointestinal complaints increase, and, at the same time, the characteristic signs of hepatic and renal involvement make their appearance. Jaundice, which is usually noted on the sixth or seventh day, rapidly increases in intensity, reaching a

peak within a few days. It is generally accompanied by enlargement and tenderness of the liver, bilirubinuria, and, occasionally, by clay-colored stools, pruritus, and bradycardia. Splenomegaly is unusual, occurring in less than 10 per cent of cases. During the initial febrile period, the urine often contains a small amount of albumin, a few cells and casts. However, as the typical renal lesion develops, the urinary output falls, albuminuria increases, casts, leukocytes, and erythrocytes appear in increasing numbers, the specific gravity tends to become fixed at 1.010, and, in the most severe cases, azotemia and anuria ensue. The blood pressure tends to fall to a low level, and a number of other cardiovascular abnormalities may be noted. Epistaxis and other hemorrhagic phenomena often occur at this time. Central nervous system manifestations are also very common, especially in seriously ill patients.

In favorable cases, signs of clinical improvement become evident as early as the second week. However, convalescence may be delayed until the third or fourth week in severe infections. Recovery is usually slow, convalescence being marked by asthenia and anemia. Relapses are relatively common, occurring in approxiately 20 per cent of cases. These are characterized by a brief recrudescence of fever and mild constitutional symptoms occurring usually between the third and fifth week. Rarely, there are several such episodes.

Most deaths occur between the ninth and sixteenth day, although fatalities have been reported as early as the fifth and as late as the twenty-first day.[41] Renal failure accounts for most of these, but hepatic failure, cardiac decompensation, massive hemorrhage, and terminal pneumonia may be important complicating factors. Only rarely is hepatic failure a primary case of death.[4] The mortality rate in *L. icterohemorrhagiae* infections varies in different parts of the world. Differences in strain virulence, individual susceptibility, and environmental factors may account for some of these variations, but the frequency with which mild infections are recognized would appear to be an important factor, since the mortality rate in nonicteric cases is negligible. In this country the mortality rate is approximately 25 per cent, which is somewhat higher than in parts of Europe where mild infections are more frequently recognized.[80]

(a) *Fever.* Characteristically, the temperature rises abruptly to 102 to 104 degrees Fahrenheit, runs an irregular remittent course, and falls to normal by rapid lysis in 4 to 7 days. Occasionally, the duration of fever is more prolonged, but rarely exceeds 12 days.[80, 45] Although a sudden onset is highly characteristic, it is not seen in all cases.[1] Severe shaking chills are

relatively uncommon. The brevity of the fever and the absence of recurrent chills are of great importance in differential diagnosis.

(b) *Jaundice.* The onset of jaundice is variable, but rarely if ever occurs before the third or after the tenth day of the disease. Characteristically, it reaches a maximum within a few days and then subsides over a period of a week or ten days. The intensity of the jaundice is variable, depending on the severity of the infection, but, in general, tends to be mild to moderate.[1] Tests of liver function usually reveal the typical pattern of hepatocellular injury.[53, 54] On the basis of current statistics it is estimated that one-third of the infections with *L. icterohaemorrhagiae* are unaccompanied by jaundice.[34] However, it has been suggested that the liver is not completely spared in such cases, since the latter often exhibit hepatomegaly[50] and impairment of liver function.[6]

(c) *Renal manifestations.* All gradations of renal injury are seen in Weil's disease. In approximately 15 per cent of cases, the kidney appears to be spared.[8] This is particularly apt to occur in patients without jaundice. However, severe renal failure has been reported in non-icteric Weil's disease, and has been termed leptospiral nephritis.[55] This condition may be mistaken for acute glomerulonephritis, although the acute onset, high fever, and myalgia should alert the clinician to the possibility of leptospirosis. The differentiation from acute pyelonephritis with renal failure may be more difficult in the absence of serological studies. Despite its severity, the renal lesion appears to be reversible and goes on to complete recovery in non-fatal cases. Leptospirae can often be demonstrated in the urine by the thirteenth to fifteenth day, and occasionally as early as the sixth day.[14] Usually they disappear by the fortieth day, but rarely may persist for many months.[55]

(d) *Meningitis.* Spinal fluid pleocytosis and xanthochromia are seen in approximately 90 per cent of patients with Weil's disease.[56] The cell count is usually under 100, but may be as high as 3,000 per cu.mm. Lymphocytes predominate, but in the first week of the disease as many as 50 per cent of the cells may be polymorphonuclear. The protein content is increased in only half the fluids with pleocytosis and is seldom high. Only half the cases with abnormal spinal fluid findings develop clinical signs of meningitis. Occasionally meningismus occurs in the absence of spinal fluid abnormalities. Leptospirae may be demonstrated in the fluid during the first week of the disease.

Meningitis is also seen in non-icteric infections with *L. icterohaemorrhagiae,*[51, 52] and may be readily confused with other forms of lymphocytic meningitis due to neurotropic viruses. Certain clinical and epidemiological features suggest that there may be some fundamental differences between

non-icteric leptospiral meningitis and that seen in classical Weil's disease. The former occurs in males under the age of 30, is seen chiefly in the late summer, and is usually associated with a normal leukocyte count and little or no muscle tenderness.[51] It is particularly apt to occur following bathing or accidental immersion in infected waters, and is often accompanied by pharyngitis, suggesting that the portal of entry may be in the nasopharynx, or possibly the conjunctivae.[52] Characteristically, the clinical course is benign and the patients are rarely very ill. An identical type of meningitis frequently accompanies *L. pomona* infections.[18, 20, 21] Rare instances of chronic leptospiral meningitis have been reported.[57]

(e) *Central nervous system.* A variety of central nervous system manifestations occur in seriously ill patients with leptospirosis. These include delirium, hallucinations, restlessness, apathy, convulsions, and loss of consciousness. Since morphological changes can seldom be demonstrated, it must be assumed that they are due to functional disturbances related to the toxemia of infection, renal insufficiency, and hepatic failure.

(f) *Hemorrhage.* Epistaxis and small conjunctival petechiae are relatively common in Weil's disease. In more severe cases, hemorrhages occur with great regularity.[39, 45] These may take the form of purpura in the skin and mucous membranes, melena, hematemesis, hemoptysis, or hematuria. Rarely, massive hemorrhage into the adrenals may produce the Waterhouse-Friderichsen syndrome.[58]

(g) *Ocular manifestations.* Characteristically, early in the course of Weil's disease there is marked injection of the conjunctival vessels, especially around the cornea, without significant conjunctival swelling or secretion. Rare complications at this stage include optic neuritis and hemorrhages into various parts of the ocular globe.

Iridocyclitis is a late sequela of leptospirosis which occurs in approximately 10 per cent of patients with Weil's disease.[5] It is also seen following infections with *L. canicola, L. pomona, L. grippotyphosa, L. hebdomadis,* and *L. australis B.*[59] The signs of uveitis usually appear one to six months following recovery from the initial acute febrile illness, but may occur as early as the second week of the disease, or as late as a year following recovery. Recent evidence suggests that the late eye complications of leptospirosis may be due to the continued presence of leptospirae in the eye. Thus, in a recently reported case of uveitis following an accidental laboratory infection with *L. alexi,*[59] leptospirae were recovered from the anterior chamber of the eye approximately seven weeks after the initial acute febrile episode. It has been suggested that survival of leptospirae in the eye may be

related to the fact that the aqueous humor, which contains little protein, has a very much lower titer of agglutinins than the serum.[59]

In a recent study of iridocyclitis carried out in Atlanta,[7] one out of the nine patients investigated was found to have serological evidence of a recent, unrecognized *L. pomona* infection. However, a survey of 102 similar cases at the Walter Reed Hospital in Washington[60] failed to disclose serological evidence of leptospiral infection in any, suggesting that leptospirosis is probably a relatively uncommon cause of uveitis in this country.

(h) *Gastrointestinal and abdominal symptoms.* Anorexia, nausea, vomiting, and constipation are frequent complaints in Weil's disease. Severe abdominal pain, tenderness and spasm, usually of muscular origin, are of particular importance, since they not infrequently lead to errors in diagnosis and needless surgical exploration for acute appendicitis or cholecystitis.[3]

(i) *Cardiovascular manifestations.* In addition to hypotension and tachycardia, which are common in many other infections, patients with Weil's disease are particularly subject to disturbances in cardiac function. Dilatation of the heart, gallop rhythm, conduction defects, arrhythmias, and electrocardiographic abnormalities are all known to occur.[56, 61] These can probably be related to the presence of myocardial lesions, although functional disturbances due to renal failure, hemorrhage, and toxemia undoubtedly play a rôle in many cases. Pericarditis is also seen occasionally.[62] All of these abnormalities appear to be reversible and leave no residuals following recovery. Rarely, leptospirae are responsible for an acute vegetative endocarditis.[5]

(j) *Respiratory symptoms.* Almost half the patients with Weil's disease develop cough and sputum, and occasionally these are accompanied by pneumonitis.[56, 60]

2. *Canicola fever. L. canicola* infections closely resemble Weil's disease, but tend to be less severe and are less often accompanied by jaundice and leukocytosis; meningeal signs and febrile relapses, on the other hand, are more common and females are affected more frequently.[6] Jaundice, which occurs in only 10 to 15 per cent of cases, is usually mild and of relatively brief duration. Meningeal signs and febrile relapses are seen in almost half the cases. The former characteristically appear early in the course of the disease, the latter during convalescence. Albuminuria is a common finding, but azotemia, hematuria, and cylindruria occur less frequently than in Weil's disease. Fatalities are rare.[44]

3. *Pretibial (Fort Bragg) fever.* The peculiar disease observed in soldiers during the period 1942-44, and later shown to be due to an infec-

tion with *L. autumnalis,* Fort Bragg,[9, 10] was characterized by an acute febrile episode accompanied by rash, splenomegaly, and leukopenia.[28] The onset was sudden, with frontal headache, post-orbital pain, backache, chills, and fever, and was characteristically followed on the fourth day by the appearance of a bilaterally symmetrical raised erythematous rash over the pretibial areas which faded in approximately 48 hours. The fever, which lasted for four to eight days, was almost always spiking in character and was usually accompanied by a relative bradycardia, slight leukopenia, and splenomegaly. Lumbar puncture in a few cases yielded normal spinal fluid. No fatalities occurred. As previously mentioned, the disease has not been encountered during the past ten years.

4. *L. pomona infections.* Most of the *L. pomona* infections in this country have been characterized by an acute febrile illness accompanied by chills, myalgia, and signs of an aseptic lymphocytic meningitis.[13, 20, 21] None has been associated with jaundice, and all have gone on to uneventful recovery. However, jaundice has been reported occasionally in swineherd's disease, the European version of *L. pomona* infection.[26] Only one American case[21] has shown the biphasic temperature curve and delayed appearance of meningitis so characteristic of swineherd's disease. In another,[19] arthritis and myocarditis were the outstanding features and suggested the possibility of rheumatic fever. Thus far only one instance of delayed iridocyclitis has been recognized.[7]

5. *L. grippotyphosa infections.* The only *L. grippotyphosa* infection recognized in this country[8] resembled classical Weil's disease and was accompanied by jaundice. In contrast, the infection in Europe tends to be milder, often resembles influenza and rarely produces jaundice.[4]

6. *L. bataviae infections.* No clinical data are available on the alleged *L. bataviae* infection reported from Louisiana.[11] In Indonesia the infection resembles Weil's disease, is often accompanied by jaundice, and has a high mortality rate.[4] However, in northern Italy the organism appears to be less virulent and seldom produces jaundice or death.[4]

VI. LABORATORY FEATURES

1. *Hematology.* The leukocyte count in classical Weil's disease is usually elevated, ranging between 12,000 and 30,000 per cu.mm., and is accompanied by an increase in the number of polymorphonuclear cells and band forms, and occasionally by the appearance of myelocytes in the peripheral blood.[5, 24] Leukocytosis is also seen in *L. canicola* infections, but much less frequently.[8] In contrast, the leukocyte count is usually normal in cases of

leptospiral meningitis, due either to *L. icterohaemorrhagiae*[51] or *L. pomona*,[30] and tends to be low in infections with *L. autumnalis*, Fort Bragg.[28]

Microcytic hypochromic anemia is often present late in the course of severe leptospiral infections.[24] This can probably be related to the loss of blood and to the hemolytic component previously described.

The platelet count and the bleeding and clotting times are usually normal.[24]

2. *Liver function tests.* In jaundiced cases the results of liver function tests are consistent with hepatocellular injury. There is usually an increase in serum bilirubin, thymol turbidity, serum alkaline phosphatase, bromsulphalein retention, and urine urobilinogen, and a decrease in prothrombin and serum cholesterol; the cephalin and cholesterol flocculation reaction is positive.[54] Bile is present in the urine, and the stools are occasionally clay-colored.[50] The prompt, direct reacting fraction of bilirubin in the serum is increased.[54] On electrophoretic analysis, the serum shows an increase in alpha-2 and gamma globulin and a decrease in albumin.[54]

3. *Demonstration of leptospirae.* Leptospirae are present in the blood during the first week of the disease, but not thereafter. They usually appear in the urine toward the end of the second week, and may persist for 30 days or more.[14] Occasionally, they can be demonstrated in the spinal fluid during the period of leptospiremia.[4]

Three methods are used for detecting the presence of leptospirae in these fluids: (1) dark-field examination, (2) culture, and (3) animal inoculation. Artefacts are so common that dark-field examination of body fluids for leptospirae is generally regarded as an unreliable procedure, even in the hands of an expert. Recently it has been shown that the direct culture technique provides the simplest and most reliable method for isolating leptospirae from blood and spinal fluid,[17] although it has the disadvantage of being somewhat slow and unsuited to the examination of urine.[63] The inoculation of susceptible animals, especially young guinea pigs, mice, and hamsters, is still the most widely used technique and is the only reliable method of isolating leptospirae from urine.[63]

(a) *Culture.* Fletcher's semi-solid medium and Schüffner's modification of Verwoort's liquid medium have both proved to be highly satisfactory for this purpose.[17]

Five ml. of medium are inoculated with 0.03 ml. of freshly drawn venous blood, or blood defibrinated by means of gentle shaking with glass beads. The latter is satisfactory for cultural purposes up to four hours following collection, so that specimens may conveniently be brought to the laboratory

when media are not immediately available.[63] The cultures are incubated in the dark at a temperature of approximately 30 degrees centigrade, and are examined by dark-field illumination on the seventh, fourteenth, and twenty-eighth days. Positive cultures can then be subcultured and identified by the agglutination-lysis antibody absorption technique. In a recent study[17] approximately one-third of the cultures were positive by the seventh day, one-half by the fourteenth day, and the remainder by the twenty-eighth day.

(b) *Animal inoculation.* Young guinea pigs, weighing approximately 150 grams, and weanling syrian golden hamsters, weighing approximately 30 grams, are inoculated intraperitoneally with 1.0 ml. and 0.5 ml. of defibrinated blood, respectively.[17] It is wise to use both species since guinea pigs are often resistant to certain strains, such as *L. canicola*[16] and *L. grippotyphosa*.[8] In the case of urine, preliminary alkalinization should be carried out by administering sodium bicarbonate to the patient before the collection is made. The specimen is then centrifuged at high speed, preferably under conditions of refrigeration, and the sediment suspended in a small volume of saline and inoculated intraperitoneally, as in the case of blood.[63] Cardiac puncture is carried out on the fourth day, and in successful transfers positive cultures are thus often obtained. Jaundice becomes apparent on the seventh or eighth day, and is usually followed by death on the tenth to twelfth day. At autopsy the characteristic findings are bile staining of the tissues and hemorrhages into the lungs, pleura, and peritoneum. However, not all infections result in jaundice and a fatal outcome, so that surviving animals should be sacrificed on the fourteenth day and cultures taken of kidney and liver suspensions in saline. If culture media are not available, serial dark-field examination of aspirated peritoneal fluid may reveal the presence of leptospirae as early as the fourth day.

4. *Serological tests.*

(a) *Agglutination-lysis.* Specific agglutinins appear in the serum during the second week. Rarely, they can be detected as early as the sixth day,[48] but diagnostic titers are usually not found before the fourteenth day. The titer continues to rise during convalescence, often exceeding 1 : 20,000, and not infrequently remains elevated for many years.[5] A titer of 1 : 300, or a fourfold increase in the titer of paired-sera from a given patient, is considered diagnostic of leptospirosis.[4, 17] The most reliable results are obtained with a freshly prepared antigen consisting of a suspension of formalinized leptospirae,[80] which has the added advantage of being safer to handle than live leptospirae. However, by using the latter it is also possible to demonstrate the presence of specific lysins in the higher dilutions of serum as the

endpoint of agglutination is reached. Following formalinization, leptospirae become resistant to the action of lysins, so that the final titer in the agglutination-lysis reaction is somewhat lower than with live organisms.[4]

As previously indicated, cross-agglutination between leptospiral strains is common, but, as a rule, the maximum titer is obtained with the particular strain involved. Nevertheless, precise identification requires cross-agglutinin absorption and neutralization tests. It has been estimated that at least seventeen antigenically distinct strains of leptospira are needed to assure detection of all known forms of leptospirosis by the agglutination-lysis technique.[17] From a practical standpoint, however, only those strains known to occur in this country need be used as test antigens.

(b) *Complement fixation.* Recently it has been shown that the complement-fixation reaction employing sonic-vibrated leptospirae as antigens is fully as reliable and specific as the agglutination-lysis technique in detecting acute leptospirosis.[17] Moreover, it has the advantage of becoming positive somewhat earlier and in requiring a smaller number of antigens. Complement-fixing antibodies may occasionally be detected as early as the second day, but more commonly they appear on about the tenth day. A four plus reaction in undiluted serum is considered positive.[68] The maximum titer attained is very much lower than that of the agglutinins, averaging only 1 to 128. Sonic-vibrated *L. icterohaemorrhagiae, L. grippotyphosa,* and *L. hyos* provide a sufficiently broad spectrum of antigens to permit recognition of the complement-fixing antibodies of most known types of leptospirosis. As might be expected, therefore, a positive complement-fixation reaction affords no information regarding the precise strain of leptospira involved.

VII. DIAGNOSIS

Of primary importance in the diagnosis of leptospirosis is an awareness of the wide distribution of the disease and familiarity with its protean manifestations. Once the possibility of infection is entertained, laboratory confirmation is relatively simple. During the first week an attempt should be made to isolate leptospirae from the blood by animal inoculation or culture. At the same time a specimen of serum should be examined for leptospiral agglutinins and/or complement-fixing antibodies. Although these are seldom demonstrable in significant titer before the end of the second week, the first determination serves as a base-line for subsequent tests which should be carried out at weekly intervals. If there is no significant rise in the titer by the end of a month, the diagnosis of leptospirosis can be excluded. Muscle biopsy and spinal fluid examination are important adjuncts, since they make it possible in some cases to establish a presumptive

diagnosis before confirmation by animal inoculation or serological study can be obtained.

During its initial phase Weil's disease may be confused with a number of other febrile illnesses, including influenza, typhus, trichinosis, brucellosis, and sepsis of various types. As localizing signs develop in the liver, kidneys, and meninges, it must be differentiated from viral hepatitis, obstructive jaundice, suppurative cholangitis, acute nephritis, and acute bacterial and viral meningitis.

The occupational history, or other opportunity for exposure to a rat-contaminated environment or to dogs, the abrupt onset, early prostration, muscle pain, and prominent conjunctival injection should alert the clinician to the possibility of Weil's disease early in the course. Later, the combination of jaundice with signs of nephritis and/or meningitis is highly suggestive of the diagnosis. Spinal fluid xanthochromia and pleocytosis are particularly important clues, since the combination is seldom seen in other types of jaundice. In severe viral hepatitis and in decompensated cirrhosis with jaundice, the spinal fluid often contains a small amount of bilirubin and an increased concentration of protein, but the cell count remains normal.[84] Rarely, spinal fluid xanthochromia is also seen in biliary obstruction, but only when the jaundice is intense and of long duration. In contrast, Weil's disease often produces xanthochromia when the jaundice is mild and of very short duration. The brevity of the febrile period, usually under a week and rarely as long as twelve days, and the infrequency of recurrent chills, except at the onset, are other important features which serve to distinguish Weil's disease from many other conditions with which it may be confused.

The cases of leptospirosis most difficult to recognize are those in which jaundice does not develop. Included in this group are not only instances of anicteric Weil's disease and canicola fever, but also most *L. pomona* infections. These may present as acute fevers without localizing symptoms, in which case the clinical features usually resemble those of the leptospiremic phase of Weil's disease, or as instances of "primary" meningitis. The possibility of leptospirosis should be considered in any case of aseptic meningitis, particularly if there has been any opportunity for exposure to rats, dogs, or cattle. Rarely, anicteric leptospirosis presents as an acute nephritis and may be mistaken for acute glomerulonephritis or pyelonephritis. The severity of the constitutional symptoms and fever are out of keeping with the former, but the differentiation from the latter may be very difficult unless the possibility of leptospirosis is considered and appropriate laboratory studies carried out.

VIII. TREATMENT

Of all the specific therapeutic agents that have been tried in Weil's disease, only immune serum of animal origin[5, 14, 35] and transfusions of convalescent whole blood or plasma[4, 14, 36] have proved effective, and, then, only if given before the appearance of jaundice. Unfortunately, anti-leptospiral serum is not available in this country, and, only under the most unusual circumstances, is a convalescent donor at hand.

The results of antibiotic therapy have been equivocal and are still under investigation. Penicillin is lethal for leptospirae in cultures and protects guinea pigs against death if given within 18 hours of infection; however, it is completely ineffective if administered following the appearance of symptoms.[66] Aureomycin and streptomycin are also lethal for leptospirae and have been shown to be therapeutically effective in dog leptospirosis, not only during the acute phase of the disease, but also in the more chronic carrier state.[66] Unfortunately, the results of antibiotic therapy in man have, on the whole, been disappointing. Penicillin appears to be effective in patients treated on the first day of illness,[38] but has no effect on the course of the disease when given after the onset of jaundice.[39] In a recent study of 67 cases penicillin, aureomycin, terramycin, chloramphenicol, and streptomycin had no effect on the duration of fever and failed to prevent or ameliorate hepatic and renal involvement.[67] Of special interest was the observation that the antibiotics frequently failed to eradicate leptospiremia and prevent relapses.[68] It should be noted, however, that treatment in this series was not begun until an average of 6.7 days had elapsed, and that 44 per cent of the cases were jaundiced. In view of its *in vitro* action on leptospirae and its effectiveness in protecting animals against fatal infections, it would seem reasonable to employ massive doses of penicillin early in the course of Weil's disease, at least until such time as the results of antibiotic therapy have been more fully evaluated.

The general management of Weil's disease is very much like that of any other acute systemic infection. However, there is, in addition, an urgent need for a close watch on kidney function, and, when renal insufficiency supervenes, for judicious regulation of fluid, electrolyte and nitrogen balance. As in other types of reversible acute renal failure characterized by severe oliguria and azotemia, the aim is to establish and maintain the internal chemical environment as close to normal as possible. This is accomplished by limiting the intake of fluid and electrolytes to measured or estimated losses, and maintaining a low protein intake while providing sufficient carbohydrate and fat to meet caloric demands. Frequent determinations of blood and urine electrolytes greatly facilitate such regulation, but are not

always essential. Blood loss is often an important consideration in severe infections and should be replaced. There is no evidence that the diet has any influence on the hepatic lesions in leptospirosis, and the high protein intake often recommended in liver disease may be distinctly harmful if renal insufficiency is present.

IX. PROPHYLAXIS

Vaccines prepared from heat-killed or phenol-treated leptospirae are effective immunizing agents.[3] They are used to some extent in areas where the disease is prevalent, and in laboratory workers who are heavily exposed to infection.

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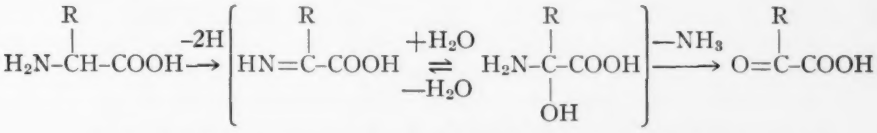
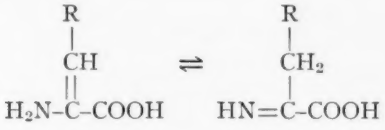
MECHANISM OF THE OXIDATION OF TYROSINE BY AMINO ACID OXIDASE OF SNAKE VENOMS†

It has been known since the studies of Neubauer in 1909 and of Knoop in 1910[7, 14] that the mammalian organism is able to degrade α-amino acids to α-keto acids. Knoop advanced the hypothesis that this degradation involved an oxidative deamination.[15] According to this hypothesis, the imino derivative of the amino acid is first formed in an oxidative step and, in turn, the imino compound yields the corresponding keto acid in an hydrolytic step. The imino acid intermediate, according to Knoop, may be in equilibrium with its hydrated form:



In 1926 Dakin[8] suggested the formation of an α,β-unsaturated amino acid intermediate during the oxidative deamination of α-amino acids. This proposal was made in analogy with Knoop's theory of the β-oxidation of fatty acids. Dakin also suggested that if such a mechanism were operative in the oxidative deamination of α-amino acids, the initial stages of amino acid and fatty acid metabolism would be essentially similar and could have certain mechanisms in common. He noted that all protein amino acids, with the exception of glycine, possess at least one hydrogen atom bound to the β-carbon of the amino acid, and recognized the possibility of a tautomeric equilibrium between the α,β-unsaturated amino acid and Knoop's imino acid intermediate:



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Bergmann and his co-workers[9] suggested, in 1925, that an α,β-unsaturated compound may be the intermediate in the reverse reaction, the physiological formation of alanine from pyruvic acid:

$$\mathrm{O{=}\overset{\displaystyle CH_3}{\overset{|}{C}}{-}COOH} \rightarrow \mathrm{HN{=}\overset{\displaystyle CH_3}{\overset{|}{C}}{-}COOH} \rightarrow \mathrm{H_2N{-}\overset{\displaystyle CH_2}{\overset{\|}{C}}{-}COOH} \rightarrow \mathrm{H_2N{-}\overset{\displaystyle CH_3}{\overset{|}{CH}}{-}COOH}$$

In 1935, Krebs[16] showed that the deamination of α-amino acids by animal tissues is indeed an oxidative reaction and that extracts of mammalian tissues catalyze the deamination of D-amino acids. The reaction is accompanied by the uptake of a stoichiometric amount of oxygen, according to the equation:

$$\mathrm{H_2N{-}\overset{\displaystyle R}{\overset{|}{CH}}{-}COOH} + \tfrac{1}{2}O_2 \rightarrow \mathrm{O{=}\overset{\displaystyle R}{\overset{|}{C}}{-}COOH} + NH_3$$

The enzyme that catalyzes this reaction was obtained in purified form by Negelein and Brömel[15] in 1939 after Warburg and Christian,[23] in the previous year, had isolated its prosthetic group and identified it as flavin adenine dinucleotide. The purified enzyme preparation, however, catalyzed an oxygen uptake (per mole amino acid oxidized) which was twice as large as that observed by Krebs. This discrepancy was explained by the presence of catalase in Krebs' preparation and the absence of catalase in Negelein and Brömel's preparation. Thus, the following schemes were found to describe adequately the experimental findings:

Catalase present:

(1) $\mathrm{H_2N{-}\overset{\displaystyle R}{\overset{|}{CH}}{-}COOH} + \text{flavin} + H_2O \rightarrow NH_3 + \text{flavin}{-}H_2 + \mathrm{O{=}\overset{\displaystyle R}{\overset{|}{C}}{-}COOH}$

(2) $\text{flavin}{-}H_2 + O_2 \rightarrow \text{flavin} + H_2O_2$

(3) $H_2O_2 \rightarrow H_2O + \tfrac{1}{2}O_2$

(Sum) $\mathrm{H_2N{-}\overset{\displaystyle R}{\overset{|}{CH}}{-}COOH} + \tfrac{1}{2}O_2 \rightarrow \mathrm{O{=}\overset{\displaystyle R}{\overset{|}{C}}{-}COOH} + NH_3$

Catalase absent:

(1) $\mathrm{H_2N{-}\overset{\displaystyle R}{\overset{|}{CH}}{-}COOH} + \text{flavin} + H_2O \rightarrow NH_3 + \text{flavin}{-}H_2 + \mathrm{O{=}\overset{\displaystyle R}{\overset{|}{C}}{-}COOH}$

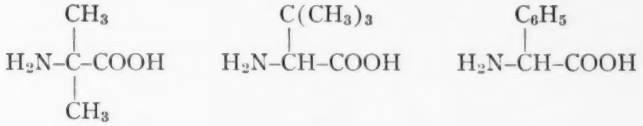
(2) $\text{flavin}{-}H_2 + O_2 \rightarrow \text{flavin} + H_2O_2$

(3) $O{=}\underset{\displaystyle R}{\underset{|}{C}}{-}COOH + H_2O_2 \rightarrow \underset{\displaystyle R}{\underset{|}{C}}OOH + CO_2 + H_2O$

(Sum) $H_2N{-}\underset{\displaystyle R}{\underset{|}{C}}H{-}COOH + O_2 \rightarrow \underset{\displaystyle R}{\underset{|}{C}}OOH + NH_3 + CO_2$

Whereas the oxidative mechanism of the deamination of α-amino acids, postulated by Knoop and Dakin, was thus demonstrated conclusively, the details of the oxidative process and the nature of possible intermediate compounds have remained obscure. Either one of the two intermediates proposed may be expected to be unstable and undergo rapid hydrolysis with the formation of ammonia and the keto acid corresponding to the amino acid substrate. It is currently and commonly assumed that the imino acid proposed by Knoop is the intermediate in the reaction.[17]

The possibility that the hydrogen on the β-carbon of α-amino acids may be involved in amino acid oxidations has, nevertheless, been considered repeatedly since the original suggestion of such a mechanism had been made by Dakin[8] and Bergmann.[3,5] Fleischer and Zeller[9] reported that α-amino acids lacking either the α-hydrogen or both β-hydrogens are not acted upon by ophio-L-amino acid oxidase. α-Aminoisobutyric acid (A), DL-β,β,β-trimethylalanine (B) and DL-phenylglycine (C) were not deaminated by this enzyme.

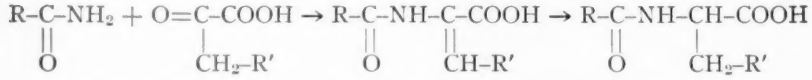


(A) (B) (C)

They suggested that the specificity patterns of two amino acid oxidases, D-amino acid oxidase of mammalian organs and ophio-L-amino acid oxidase, agree with the hypothesis of Bergmann and Dakin.

Indirect support for the participation of dehydroamino acids in intermediate metabolism has also been forthcoming in the past. Because of the low stability of the dehydroamino acids, work on the possible significance and presence of these compounds in biological systems had to be restricted to their stable acylated derivatives. Bergmann and Schleich[4] discovered an enzyme of rather wide distribution in animal tissues[11] which specifically hydrolyzes peptide bonds involving dehydroamino acids. It was also sug-

gested by Bergmann and Fruton[1] that dehydropeptides may be formed *in vivo,* in analogy with the *in vitro* reactions of keto acids with acid amides,[2, 20] followed by a reduction of the resulting acetyldehydroamino acid, thus representing a possible path of peptide biosynthesis:



Fruton, Simmonds, and Smith[10] have shown that several acetyldehydroamino acids are metabolized by growing cultures of *Escherichia coli.* These studies have shown that derivatives of dehydroamino acids may have a rôle in metabolic reactions.

EXPERIMENTAL

The present study was undertaken in an attempt to test the hypothesis that dehydroamino acids are formed during the enzymatic oxidative deamination of amino acids. From the findings of Fruton, Simmonds, and Smith,[10] who observed that the characteristic absorption bands of N-acetyldehydrotyrosine exhibit a marked shift in wave length and increase in intensity when compared with the ultraviolet absorption spectrum of N-acetyltyrosine, it may be expected that similar bathochromic and hyperchromic effects will manifest themselves when α,β-unsaturation is introduced into the free amino acid. The conjugation of the introduced chromophore with the resonating aromatic ring should result in a comparable electronic interaction in either system, manifesting itself by a much more intense benzenoid absorption shifted to longer wave lengths.[8] Thus, the differing absorption behavior of tyrosine and its dehydro derivative was taken as the experimental basis of the work which is the subject of this report. Ophio-L-amino acid oxidase was selected because of its ready availability and its high potency in catalyzing the oxidative deamination of aromatic amino acids.[24]

A Beckman Model DU Spectrophotometer was used for all optical density measurements. Silica cells with a 10 mm. light path were used. Measurements of optical density changes during the enzymatic reaction were, in general, carried out by mixing the components in a silica cell and placing the cell into the cell compartment of the instrument. Optical density readings were then taken at known times after the mixing of substrate and enzyme preparation. The instrument was usually set for 100% transmission with buffer solution in the cell. It was ascertained that no two components affected the light absorption of each other except a mixture of tyrosine and the enzyme preparation. These tests included incubation of *p*-hydroxyphenylpyruvic acid with venom, catalase, and ammonium chloride as well. Mixing of the keto acid with any one or any combination of these components resulted in optical density readings close to values expected on the basis of additivity of the optical densities of the individual components. All such readings were independent of the time after mixing. The solutions employed in each experiment were freshly made up except the enzyme solutions. These were stored in the refrigerator and may have been a few days old when used. Oxygen uptake measurements indicated no loss of activity resulting from such storage. Determinations of enzyme activity were made by means of the conventional Warburg manometric apparatus for measuring oxygen uptake.

Ophio-L-amino acid oxidase was obtained by following exactly the procedure of Singer and Kearney[21] for its purification from moccasin venom (*Agkistrodon piscivorus*). Crude venom solutions or a preparation which was purified 15-fold were employed in all experiments. No differences were observed between optical density changes catalyzed by either enzyme preparation.

The phosphate buffer employed was prepared by mixing the appropriate volumes of a KH_2PO_4 and of a K_2HPO_4 solution (0.05 M). The buffer contained also KCl (0.06 M) in order to protect the enzyme from spontaneous inactivation, according to the observations of Kearney and Singer.[18]

L-Tyrosine was a commercial preparation (Eastman) and was used without further purification. N-Acetyldehydrotyrosine was prepared by the procedure of Bergmann and Stern.[5] *p*-Hydroxyphenylpyruvic acid was prepared by a slight modification of the procedure of Herbst and Shemin[19] for the preparation of phenylpyruvic acid. At the end of the acid hydrolysis of acetyldehydrotyrosine the hydrolysate was treated with Norite, and from the hot filtrate a good crystalline product was obtained. Synthetic compounds were identified by their melting points and mixed melting points with authentic samples.

Bothrops atrox venom and *Agkistrodon piscivorus* (moccasin) venom were obtained from Ross Allen's Reptile Institute, Silver Springs, Florida. Catalase was obtained by centrifugation of a crude commercial preparation (Armour). The clear supernatant fluid was used.

RESULTS AND DISCUSSION

The ultraviolet absorption spectra of tyrosine, acetyldehydrotyrosine, and *p*-hydroxyphenylpyruvic acid were obtained in a phosphate buffer containing KCl, pH 7.29, with points at intervals of 20*A* (Fig. 1). It will be seen that under these conditions acetyldehydrotyrosine exhibits maximal absorption in the region of 290 mμ. For this reason, this segment of the spectrum was selected for a study of the change in the absorption of a solution containing L-tyrosine and snake venom preparation (*Bothrops atrox*, selected for its reported high activity on tyrosine[24]). Initial experiments revealed a rapid rise in the optical density of the solution after mixing. These measurements were made at wave lengths between 290 and 310 mμ; 300 mμ was finally selected for subsequent experiments as the wave length at which the most marked optical density changes could be observed. It was established that changes in optical density were dependent on oxygen uptake by bubbling oxygen and nitrogen, in turn, through the cell contents. It appeared desirable to perform subsequent systematic experiments in the presence of catalase, thereby stopping the reaction at the *p*-hydroxyphenylpyruvic acid stage without further decomposition of the product. Subsequent experiments were also performed using a purified enzyme preparation from *Agkistrodon piscivorus* (moccasin) venom.

A series of experiments was performed to determine whether changes in the optical density of substrate-enzyme solutions could be accounted for in terms of concentration changes of substrate (tyrosine) and product (*p*-hydroxyphenylpyruvic acid) alone. The optical density of a solution is equal to the algebraic sum of the extinctions due to its various components: (D = optical density, c = molar concentration, ϵ = molar extinction coefficient, t = tyrosine (suffix), p = hydroxyphenylpyruvic acid (suffix), i = initial (suffix), T = time)

$$D_1 + D_2 + D_3 + \ldots + D_n = D$$
$$D = c \cdot \epsilon$$
$$c_1\epsilon_1 + c_2\epsilon_2 + c_3\epsilon_3 + \ldots + c_n\epsilon_n = D$$

If it is assumed that the optical density of the solution changes with time only because the concentrations of tyrosine and *p*-hydroxyphenylpyruvic acid and of no other compounds change, then:

$$\frac{dD}{dT} = \epsilon_t \frac{dc_t}{dT} + \epsilon_p \frac{dc_p}{dT}$$
$$c_{ti} = c_t + c_p = \text{constant}$$
$$\frac{dD}{dT} = \frac{dc_p}{dT}(\epsilon_t + \epsilon_p)$$

or, more correctly, since the concentration of tyrosine decreases as the concentration of *p*-hydroxyphenylpyruvic acid increases:

$$\frac{dD}{dT} = \frac{dc_p}{dT}(\epsilon_p - \epsilon_t)$$

If the change of optical density with time is followed at two different wave lengths, the ratio of the rates of these changes must be equal to the ratio of the differences of the respective molar absorption coefficients, which is a constant:

$$\frac{\frac{dD_{\lambda 1}}{dT}}{\frac{dD_{\lambda 2}}{dT}} = \frac{\frac{dc_p}{dT}}{\frac{dc_p}{dT}} \times \frac{(\epsilon_{p\lambda 1} - \epsilon_{t\lambda 1})}{(\epsilon_{p\lambda 2} - \epsilon_{t\lambda 2})} = \frac{(\epsilon_{p\lambda 1} - \epsilon_{t\lambda 1})}{(\epsilon_{p\lambda 2} - \epsilon_{t\lambda 2})}$$

In Figure 2 the data obtained in such an experiment are shown graphically. The wave lengths were selected after inspection of the data shown in Figure 1. It is apparent that the ratio of the rates of the optical density changes is not constant throughout the experiment. The ratio $\Delta D_{246}/\Delta D_{300}$ varied between 1.6 at 0.5 min. and 2.4 at 4.0 min. It follows from these data that if Beer's law is obeyed by tyrosine and *p*-hydroxyphenylpyruvic

acid at the given concentrations, then the spectrophotometric results cannot be accounted for by the formation or disappearance of *p*-hydroxyphenylpyruvic acid and tyrosine alone. Rather, the results indicate the formation of at least one more molecular species. Furthermore, it may be suggested that if this additional molecular species is being formed more rapidly during

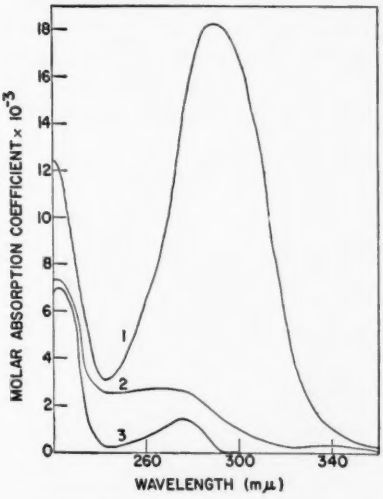


FIG. 1. The U.V. absorption spectra of tyrosine, acetyldehydrotyrosine, and *p*-hydroxyphenylpyruvic acid. All compounds were dissolved in 0.05 M phosphate buffer (pH 7.23) containing KCl (0.06 M). Curve 1, acetyldehydrotyrosine; curve 2, *p*-hydroxyphenylpyruvic acid; curve 3, DL-tyrosine.

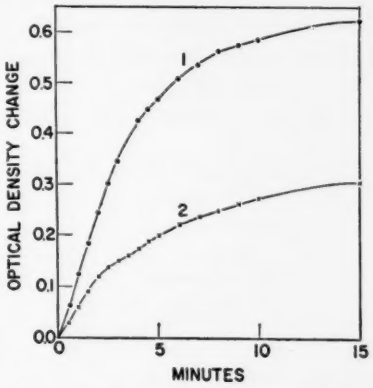


FIG. 2. Optical density vs. time at 246 mμ and 300 mμ in tyrosine-purified moccasin venom—catalase system. Reference cell: 1.3 ml. 0.05 M phosphate buffer (pH 7.22) containing KCl (0.06 M). Reaction cell: 1.0 ml. 1.3 mM L-tyrosine solution (in above buffer), 0.2 ml. purified enzyme solution (equivalent to 1 mg. of crude venom based on O_2 uptake measurements), 0.1 ml. catalase solution. Enzyme was added at zero time. Optical density values plotted represent increase over the sum of the optical densities of tyrosine and enzyme solutions at the concentrations at which they were present in the reaction cell at zero time. Curve 1, 246 mμ; curve 2, 300 mμ.

the very early stages of the reaction, building up toward a steady state concentration, then the data are consistent with the formation of a compound absorbing more strongly at 300 mμ than at 246 mμ.

In considering the significance of the data, attention must be given to some details of the experimental procedure. It was necessary to follow the optical density changes at the two wave lengths in turn, involving the use of different but identically prepared reaction mixtures. In every instance, two time curves of optical density changes were obtained immediately, one

after the other. The room temperature at which the measurements were made was constant during a set of measurements within 0.2° C. The solutions were allowed to equilibrate with the room temperature. Duplicate experiments yielded data agreeing within 0.004 optical density units at corresponding times after mixing. It was found that reproducible data could be obtained if the components of the reaction mixture were mixed by stirring for a definite length of time and if the cells were left uncovered without any further attempt to control the oxygen uptake by the solution. Bubbling of a stream of air through the solution between readings did not lead to reproducible data. In view of the very satisfactory agreement of duplicate experiments in which optical density changes at the same wave length were

TABLE 1. EFFECT OF HYDROGEN PEROXIDE ON THE OPTICAL DENSITY OF A *p*-HYDROXYPHENYLPYRUVIC ACID SOLUTION.

Conditions: reference cell: 1.3 ml. 50 mM phosphate buffer, pH 7.22, containing 60 mM KCl; reaction cell: 1.0 ml. 0.65 mM *p*-hydroxyphenylpyruvic acid solution (in above buffer), 0.2 ml. buffer, 0.1 ml. 13 mM hydrogen peroxide solution.

Time (minutes)	0	1	2	5	10	15	20
Optical density (300 mμ)	0.486	0.472	0.463	0.443	0.420	0.396	0.374

measured, it is reasonable to assume that the reproducibility of the reaction rates was just as satisfactory when the optical density changes were followed at different wave lengths. Hence, the difference between ratios of 1.6 and 2.0 or larger must be considered significant. It was also found that if the concentration of enzyme preparation was varied, the 246 mμ/300 mμ ratio of optical densities was lowered with increasing enzyme concentration. Thus, the concentration of the substance absorbing preferentially at 300 mμ must have increased if the enzyme concentration was increased.

A second series of experiments provided support for the suggestion that while the optical density increase at 246 mμ during the reaction is mainly due to the formation of *p*-hydroxyphenylpyruvic acid, the optical density increase at 300 mμ, especially during the first few minutes of the reaction, is due to a compound different from *p*-hydroxyphenylpyruvic acid. It was found in a control experiment that if hydrogen peroxide was added to a *p*-hydroxyphenylpyruvic acid solution, the optical density at 300 mμ of such a solution decreased steadily with time (Table 1). The effect of the addition of hydrogen peroxide to the usual reaction mixture containing tyrosine and enzyme was now observed by measuring optical density changes at 300 mμ

and at 246 mμ. The portions of interest of the time curves obtained are reproduced in Figures 3 and 4. In Figure 3 the optical density changes measured at 300 mμ are summarized. It is apparent that until two minutes have elapsed after the mixing of the components, the presence of hydrogen peroxide, formed by the reaction itself (no added catalase or peroxide, Curve A) or added to the reaction mixture (Curve B) at zero time, shows no spectrophotometrically detectable effect on the rate of the reaction when

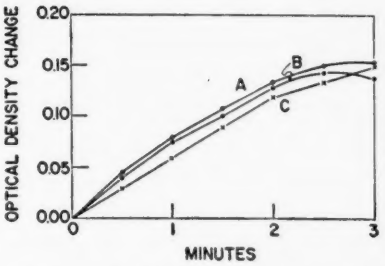


FIG. 3. Optical density changes at 300 mμ in the absence and presence of hydrogen peroxide. Conditions are identical with those of the experiment represented in Figure 2 except that the 0.1 ml. of catalase solution was replaced by either 0.1 ml. of buffer or 0.1 ml. of 0.013 M H_2O_2 solution, as indicated. Optical density values plotted represent increase over sum of optical densities of tyrosine, amino acid oxidase and catalase, or H_2O_2 solutions at the concentrations at which they were present in the reaction cell at zero time. O, no additions; •, H_2O_2 added; x, catalase added.

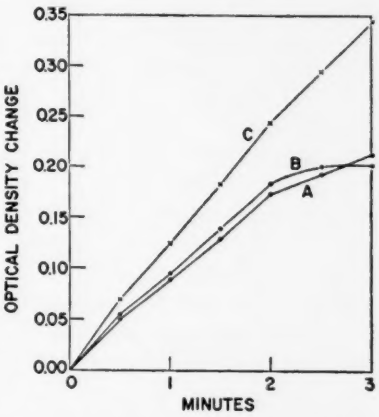


FIG. 4. Optical density changes at 246 mμ in the absence and presence of hydrogen peroxide. Conditions are identical with those given in Figure 3.

compared with the reaction curve of the system containing catalase (Curve C). In view of the results of the control experiment (Table 1) it appears that the optical density increase at 300 mμ during the first part of the reaction, as given in Figure 3, is due in large measure to a substance different from the keto acid. The definite breaks in the slopes of curves A and B at 2½ minutes, continuing beyond the 3 minutes shown on the graph, could be due to a leveling-off in the concentration of this compound while the product, *p*-hydroxyphenylpyruvic acid, increases in the presence of catalase (Curve C) or is decomposed to the less strongly absorbing *p*-hydroxyphenylacetic acid by the hydrogen peroxide formed by the reoxidation of the reduced flavin by molecular oxygen (Curve A), or is decomposed at an even faster rate by the added peroxide (Curve B). Figure 4 summarizes

the optical density changes measured at 246 mμ. It is apparent that, from the start of the experiment, there is a definite difference in the rates of the optical density changes measured in the presence of catalase or in the presence of peroxide. Employing the same reasoning as in the interpretation of Figure 3, it may be inferred that the optical density at this wave length is probably largely due to the keto acid, which is being transformed into a less absorbing compound by hydrogen peroxide. The reproducibility of these results was found to be as satisfactory as that of the data obtained in the previous experiments. It should be noted that the addition of hydrogen peroxide introduces a compound which absorbs light in the ultraviolet region, but the extinction coefficients of H_2O_2 at the wave lengths employed[19] and the concentration changes involved (1 mole of hydrogen peroxide destroyed per mole of *p*-hydroxyphenylpyruvic acid decomposed) are such as to leave the large optical density changes observed relatively unaffected. It is also known that U.V. irradiation of a hydrogen peroxide solution in the Beckman DU spectrophotometer causes no decomposition.[20]

It should be added that whether crude *Bothrops atrox* venom, crude moccasin venom, or purified moccasin venom was employed as the enzyme source, the corresponding data were qualitatively the same. Quantitatively, the results with moccasin venom show smaller effects but this would be expected since the L-amino acid oxidase of *Bothrops atrox* venom is known to be especially active toward tyrosine.[24] It should also be noted that the spectrophotometrically observable effects followed the purification of the enzyme quantitatively. Nearly identical curves were obtained using crude moccasin venom or a purified enzyme solution in amounts which catalyzed the same amount of oxygen uptake in the Warburg manometric apparatus.

Although these data offer support to the hypothesis that the enzymic oxidation of L-tyrosine by ophio-L-amino acid oxidase involves the intermediate formation of an α,β-unsaturated compound, definite proof for this view requires further evidence. This is being sought in experiments designed to demonstrate the labilization of the hydrogen at the β-carbon of the amino acid. Since α,β-unsaturated amino acids are known to be unstable in aqueous solution, it may be surmised that if such a compound is formed in the reaction under study, it may be partially stabilized by its attachment to the enzyme protein.

SUMMARY

Spectrophotometric data have been presented to show that, in addition to L-tyrosine and *p*-hydroxyphenylpyruvic acid, at least one molecular species is formed when L-tyrosine is incubated with ophio-L-amino acid oxidase in

the presence of catalase. The concentration of such a compound (or compounds) appears to increase rapidly after mixing of enzyme and substrate and to level off within a few minutes. This compound (or compounds) exhibits characteristic light absorption in the 300 mμ region more intensively than in the 246 mμ region. It is proposed that the intermediate formation of the α,β-unsaturated amino acid in the oxidative deamination of L-tyrosine by ophio-L-amino acid oxidase would be consistent with the experimental data presented.

ACKNOWLEDGMENTS

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The Board of Editors

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THE CONTRIBUTION OF THE STUDENT TO EDUCATION†

The opportunity to present this lecture has provided the motivation to speculate and to concretize some concepts concerning the characteristics, significance, and importance of faculty-student relationships; or, with proper acknowledgment to the school of general semantics, this might be more precisely stated as: $Student_A$ - $Student_B$ relationships.

In the course of this speculation some startling but rather simple observations became apparent. The most significant of these, I believe, was the recognition of the importance of the *student* to the faculty. This may seem obvious, and it is in certain respects. However, the contribution made by the student to the faculty, concerning which I should like to call your attention this evening, is related directly to your participation in continuing education; and, perverse as it may sound, this contribution frequently is related inversely to the quantity of material we teach.

In order to be meaningful, an educational experience must be implemented in a system that involves two-way communication. The educator (i.e., the faculty) sends a message which the student receives and interprets. A variety of distortions may be introduced at several points in the total communication system. A common source of distortion is the inability of the sender to utilize the machinery of communication in an effective manner. The dispatcher may have difficulty in formulating the message appropriately, or may be hampered by a lack of data. The message may be distorted by difficulties associated with the characteristics of the transmitting media. Lastly, distortion may be associated with the receiving instrument; in this instance, the student. If these distortions are to be eliminated, the dispatcher must be aware of the nature of the reception, otherwise he will transmit in a vacuum with no conception of whether his message is being clearly received or badly distorted. There are a variety of signals which serve to inform the dispatcher, and among these may be mentioned the degree of your enthusiasm, your habits of thought, your general conduct,

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* Professor of Medicine.

† This lecture was presented on September 15, 1954 as the Whitehead Lecture, sponsored by the student organization named the Whitehead Society in honor of the first dean of the University of North Carolina School of Medicine. The lecture is presented each year as a part of the orientation exercises for the students of the School of Medicine.

and your answers to questions. However, the signals that are best adapted to indicate distortions are your *questions* directed to the faculty. These signals may make it readily apparent that the message was poorly constructed, or that an improper or inadequate medium was used to send it, or that there was something wrong in the receiving set that interfered with your ability to receive, record, and interpret it appropriately. In this very simple fashion the students are able to share part of the responsibility for their education. The communication system is poor indeed if messages cannot be received and interpreted properly in a reasonably consistent fashion.

More important, however, is the signal which tells the faculty that the message as sent was received "loud and clear," but that despite this was incomprehensible. This usually means either: (1) that the message was valid but could not be interpreted because some part of the receiving set was missing or poorly connected, or (2) that the message was substantially invalid despite the excellence of its mechanical transmission and reception. The signal which indicates that the message was heard "loud and clear" but that it was incomprehensible is usually sent in the form of a critical and searching question by the student. It is then incumbent on the thoughtful teacher to re-evaluate the message in terms of its essential comprehensibility, specifically, with reference to the particular nature of the receiving set at the other end of the communication system. He may be able to reconstruct the message so that it can be interpreted; or he may find that a minor adjustment made in the receiving set—perhaps, by the addition of a single datum—will make the original message comprehensible or he may decide, after due reflection, that it was basically invalid.

Each of us who functions as a dispatcher in our educational system has been working with certain problems in a particular area for some time. It is inevitable that we will collect and retain a number of preconceptions, that we cease to think critically about certain concepts, or fail to recognize that new data have invalidated our ideas. You will find that we may provide glib and oversimplified explanations of medical phenomena. In contrast, you students approach these problems with fresh eagerness and with an unprejudiced orientation; you will not, as yet, have developed emotional attachments to pet concepts. You are not bound by traditional patterns of thought and, hence, on many occasions, you will find that the explanations that we have considered satisfactory for some years are completely unacceptable. If you challenge our hypotheses with intelligent criticism, we are forced to review and re-evaluate all of the available data. On some occasions this analysis will serve to reinforce our acceptance of the challenged

hypothesis but it may provide insight into a better way to send the message. However, more often than you suspect, the review of all the data may make it quite clear that the concept is, in fact, untenable, and hence rightfully uninterpretable by you. Your critical analyses expressed as doubts and questions raise new problems that must be resolved. In this fashion the faculty benefits in a remarkable manner from the two-way communication system; new questions, or old questions restated, become the substrate for a series of investigations which ultimately must increase our insight into basic physiological mechanisms and the manner in which alterations in these mechanisms impinge on the total response of the organism which is the expression of a disease.

This insight into the nature of the relationships between student and faculty has other significant implications. Perhaps the most important of all for you to recognize is that, unlike the multiplication tables in arithmetic, the biological and medical sciences represent a *process*. There is constant change and growth, and our ignorance is abysmal. Hypotheses which were reasonable in the nineteen-thirties are unacceptable in whole or in part in the nineteen-fifties. This does not mean that those of us who had our introduction to the study of medicine in the nineteen-thirties wasted our time in the attempt to understand these earlier hypotheses. To the extent that they represented the best approximate resolution of the scanty data that were available at that time, the old hypotheses were useful in our thinking about a problem. More important is the fact that they supplied the framework within which new questions were formulated. When the experimental data which were gathered to answer the new questions were found to be inconsistent with the initial framework, the hypothesis had to be altered or destroyed and new concepts substituted. The current hypotheses derived by this process will serve as the framework within which you will experience your introduction to the study of medicine. The phrase "introduction to the study of medicine" should be emphasized, for it is implicit in what has been said that in many instances what you are about to learn is not the ultimate answer. It is therefore most important that you learn *how to learn*. If you accept uncritically all that you are exposed to in the four years of the medical curriculum and fail to understand that these concepts are merely the best tentative syntheses that can be offered at this time, your education will be sadly dated the year after you graduate. In contrast, if you will understand that new data must modify your concepts, and that this process of change will continue forever, and if you are prepared to remain *au courant* with these changes, your education will, likewise, continue forever. Although this understanding is more important than

the details you will be required to master, you must not misinterpret these remarks to mean that it is useless and unnecessary to study today's concepts. The synthesis of tomorrow's hypotheses will always have today's ideas as the base of reference; and the ease with which you will be able to understand the implications of new data will be closely related to your understanding and appreciation of the merits and inadequacies of the older concepts. This view of the rôle of the student in medical education has been well stated in a recent report published by the World Health Organization: "The time which a student spends in a medical teaching institution must be viewed as a phase which is affected by what has passed before and which, in turn, will influence what is yet to come. In this sense, the student may be considered as the principal vehicle by which medicine is projected into the future."

If the student is to understand today's concepts, he must expose himself to more than one educational technique. The lecture can be made a useful tool in education when it complements your reading and study. It can serve to present individual points of view and philosophical attitudes. It can be most effective if the lecturer can assume that you have already familiarized yourselves with at least some of the standard literature. He is then in a position to aid you in the interpretation and synthesis of what may appear to be irrelevant and conflicting data. In the process of your own reading and study you will prepare the basis that is indispensable for the reception of the lecture message. Your curiosity should lead you to a literature with which the lecturer cannot possibly cope in a brief period. However, if you present him with the opportunity, he may be able, in the same short time, to help you with an interpretation that may clarify several hours of your investment in study. The lecture really only helps you to an extent commensurate with your individual pursuit of information and understanding; and in the absence of this effort on your part, the "chalk and talk" exercises will make little or no contribution to your education.

The student, if he wishes, can readily participate in the research that his questions have stimulated. There are many opportunities, and a student will always find a cordial welcome should he express the desire to help in the search for answers to questions. An experience in a research investigation is most gratifying and is a powerful educational tool. Its values far exceed the merits of the specific data that may be acquired. It provides an opportunity for a more intimate and mutually beneficial relationship between the student and individual members of the faculty. It reinforces the principles of the scientific approach which have general application in all medical activities. It provides insight which helps to develop a critical atti-

tude toward the great mass of literature to which we are all constantly exposed. These values are not abridged by the modest proportions of the research, nor should the lack of an immediately practical application serve as a deterrent. Dr. Richard Henry Whitehead, whose name honors your Society, presented his thoughts on these matters in the presidential address before the Philosophical Society of the University of Virginia in 1915. I should like to quote a small portion of his remarks:

"We need not worry very much about the immediate 'practical' value of research. If history teaches anything, it is that a given generation is by no means always the best judge of the work done or the facts brought to light during its brief existence. A monk makes certain experiments upon some flowers in the monastery garden, and his observations attract no attention from his contemporaries; but fifty years afterward these same observations become the basis of one of the most interesting and fruitful of modern hypotheses. Does anybody suppose that Louis Pasteur as he watched the behavior of certain crystals dreamed that he was taking the first step in the creation of a new science which would transform medical thought and practice and bring untold good to mankind? And who fifty years ago could possibly have foreseen that the completion of the Panama Canal would be conditioned upon a knowledge of the natural history of a wretched insect? And so when that eternal question, 'What's the use,' asks itself, as it so often will, we can leave the final answer to those who shall come after us. . . . The essential thing is that the worker shall be actuated by a strong desire to know the truth, so that the work will be honest, and the workman have joy in his work. If in this spirit a man finds a bit of truth, or approximation to the truth, however modest, his work is good—good for him and good for those submitted to his influence."

I thought this was a most important concept extremely well expressed.

Next, I would warn you against the philosophy of the "practical" in your general medical education. This philosophy takes the view that the training of a physician should be limited to those items which can be clearly characterized as having immediate values in the management of the ill. If one examines this concept carefully, it will become quite obvious that this is a most impractical point of view. Overemphasis on "facts" and techniques and under-emphasis on broad general principles can hardly be honored with the phrase "medical education"; and as technical training it will, at best, be reasonably valid only during the few years during which you receive it. It is an anti-intellectual attitude which does not foster a spirit of scholarship. The area of medical education which is criticized and interfered with to the greatest extent by those who support this philosophy consists of the basic preclinical sciences. Some would say that too much time is spent in these disciplines. It is suggested that the medical student should begin to see and experience contact with patients on the very first day of matriculation.

The fabulous growth in medicine which is apparent to all of us is directly dependent on the scientific attitudes taught and the data collected by the methods of the preclinical disciplines. More emphasis, not less, must be placed on the study of physiological, biochemical, pathological, and psychological mechanisms. The consideration of disease processes, symptoms, signs, and therapy cannot be properly appreciated without rigorous attention to basic mechanisms and their alterations which are the expression of a clinical disorder. It is in the basic preclinical sciences that the scientific methodology and approach can best be inculcated in the new student.

It is suggested, for example, that the importance of the study of acid-base relationships can *best* be illustrated to the first-year student by having him see a patient with diabetic acidosis. Certainly there is something more profound in the study of these matters than such an immediate and narrow application. An understanding of these problems as they may be related to the broad study of physiological reactions will allow the student to be free to use this information in a variety of circumstances as he ultimately meets them at the bedside. Such a specific orientation and subversion of a major group of physiological and chemical inter-reactions would appear to be a shortsighted exploitation of a basic science. The study of these basic mechanisms needs no such puny motivation—these studies have intrinsic merit and a dignity which demand no further defense.

There are other overtones in the "practical" approach that are disturbing. There is the inherent danger that the student will consider the basic sciences as a hurdle in his path to the bedside, rather than as the very important substrata upon which his clinical experience must be imposed if the latter is to have any lasting significance. Moreover, I would suggest that the opportunity for the student to share in the responsibility of the care of a patient is a privilege that should be earned, rather than an exercise which is thrust upon him prematurely.

Another group recommends that correlation among the several divisions of medical science must begin at once. Correlation is certainly a most important function of education. However, the act of correlation implies that there has been a preliminary study of data which can be correlated. It is certainly important to understand that the cell is not simply a bag of enzyme systems, but that cell structure makes it possible for these enzyme systems to engage in a coordinated series of productive reactions. It would appear logical, however, that some understanding of an enzyme system, and some knowledge of cell structure must be prerequisites for the correlative attempt. Correlation will be more effective and meaningful if you are

allowed some little time to acquire an understanding of the basic data and concepts.

Lastly, I should like to comment on the damaging influence of fear on the part of the student. In other schools and other times it was the custom for the dean to point out to an entering class that a fair number of the students would not complete the curriculum. A favorite statement, for example, was that the student should look at the men sitting on either side of him and if they graduated he would not. This was a reflection of the studied practice of admitting more students than the number that were expected would continue beyond the first year. Very few, if any, schools have continued this practice. The selection is made on the basis that each student admitted is well motivated to be a physician and has the intellectual equipment to contend with the material. It is certainly not anticipated that any student will be forced to leave for reasons of scholastic failure. I should like to emphasize this point because fear will impose restraints which may make you unwilling to ask questions or participate freely in discussions; it will jeopardize your performance in oral or written examinations, and it is certainly not conducive to a sound educational experience. If you depend on fear as the motivation to study and understand the problems you will meet, your information will tend more to be memorized than understood and you will have very little of the fun and excitement which should be yours.

The Dean's Office undertook a research problem for us and analyzed the experience of students who have entered this medical school as freshmen since 1942 in an effort to see how many failed, scholastically, to complete the requirements for the degree of doctor of medicine. There were only 53 of a total of 709 who could be placed in this category; and it might be worthwhile to mention that the preclinical departments at this school have unusually high standards. Until two years ago, when it was no longer necessary, our students transferred to some of the best medical schools in the country for their clinical work. If one excludes the years when the Armed Forces dictated many admissions, there were only 18 of 411 who failed. Thus, the chance that any individual freshman will not become a physician because of scholastic failure is about one in twenty. There is nothing of any consequence that you will ever undertake in your life that will have as good or better odds for success than 20 to 1. These data should reassure you. Barring illness and other acts of God, you will be a physician. You have no reason for fear and, hence, you can utilize your full energies toward acquiring methods of thought and learning, as well as information, and enjoy yourself tremendously in the process.

Dr. Hedgpeth once told me that there were two types of lecture. In one group the lecturer showed lantern slides and in the other the speaker had a message. I have taken his advice quite seriously and you will note that I have scrupulously avoided the use of a single chart or graph because I wanted to bring you a message. In summary, these are the thoughts I would leave with you:

1. You are not merely the receptors in an educational process. You must also serve as the senders of signals and messages. Education is, indeed, a two-way system of communication. Listen and read attentively, but do not mistake authority for truth and do not submit to authority in lieu of a more rational explanation of conflicting data.

2. Cultivate the techniques of *learning* as assiduously as you attempt to master "facts." The latter are always in quotes; they have a very temporal quality, and they are important as hypotheses only insofar as they are helpful in understanding today's data, and as they serve as the base of reference to gain more information and better insights. These latter may then destroy the initial hypothesis itself.

3. Accept some of the challenges your questions offer and seek the opportunity to share in an investigational experience. Although it may increase your investment of time, the rewards make it profitable.

4. Do not consider the preclinical sciences as an obstruction in your path to the bedside. These are truly the basic medical sciences, and without a proper understanding of these your appearance at the bedside will be an irrelevant event. The *sine qua non* of the fine physician is a highly developed sense of responsibility. Your first responsibility is to prepare yourself properly for the study and care of the ill.

5. Lastly, consider your faculty as your fellow students and your friends. We expect to help you to learn, and, in turn, you must contribute to *our* education. To the extent that you fail to do this, we will be a poorer faculty, and you will ultimately suffer thereby. Our ability to help you and those that follow is contingent on your contribution as students, and we hope that you will remain students throughout your careers in Medicine.

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EASTERN EQUINE ENCEPHALITIS IN CONNECTICUT: A SEROLOGICAL SURVEY OF PHEASANT FARMERS†‡

In the summer of 1938 human cases of acute encephalitis appeared in New England in sufficient numbers to warrant designating the outbreak as an epidemic.[4] In Massachusetts this epidemic involved more than 30 persons, mostly children, with a mortality rate of almost 75%. In the same area and at the same time, 90% of some 250 local horses which had encephalitis died. The causative virus of eastern equine encephalitis was first recovered in this epidemic from human tissues of the central nervous system by Fothergill *et al.*,[5] and by Webster and Wright.[13] This was accomplished from specimens of brain tissue of patients hospitalized in Boston. During the fall of that year the disease was detected in pheasants and in a pigeon caught within the same area.

As a result of experiments with the causative virus, Ten Broeck decided that from the epidemiological standpoint birds are more likely than horses to act as reservoir hosts.[12] He also demonstrated that birds might have a transient period of viremia without apparent signs of infection.[12] The same investigator marshalled considerable experimental evidence to indicate that *Aedes* mosquitoes might be considered as natural vectors for this disease. At about this time Davis[3] reported important observations on the ease with which birds may be infected with eastern equine encephalomyelitis virus and develop viremia and often serious lesions.

The extent of the New England epidemic in 1938 was never actually determined, but it was probably more widespread than was realized at that time. During that same year a number of cases of unidentified acute

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† With the technical assistance of Manuel Ramos, Dorothy Davis, and Betty E. Bryan.

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"encephalitis" were seen in south central Connecticut, mostly in Southington although a few in Cheshire. One fatal case from Cheshire, Connecticut, was admitted to the New Haven Hospital in September 1938, in which both clinical and autopsy findings were compatible with the diagnosis of acute viral encephalitis. In spite of vigorous efforts, no virus was isolated from the brain tissue collected at autopsy.[9] During the intervening 16 years, i.e. since 1938, reports of human cases of this disease in New England have been scanty or practically nil. Although the diagnosis has been suspected in a few "summer cases of encephalitis" which have been seen at the Grace-New Haven Community Hospital, isolation of the virus or efforts to demonstrate appropriate antibodies have not been successful.[10]

Elsewhere in the United States there have been occasional, sporadic cases of the human disease. In 1947, in Louisiana, an explosive epidemic involved 14,000 horses of which 90% died, and there were about 15 human patients of whom 9 died.[8]

Since 1938 much has been learned about the epidemiology of the various members of the arthropod-borne virus encephalitides, particularly with regard to their hosts and insect vectors.[6, 11] Nevertheless, insect vectors for the transmission of eastern equine encephalitis virus have not yet been adequately defined and certainly there is little or no local knowledge as to what they might be in Connecticut. From studies made elsewhere there is more than a suspicion, however, that several species of mosquitoes may be involved. It also seems reasonable that this infection may occasionally or frequently spread from bird to bird, or from birds to mammals, by some means other than the bite of a mosquito, i.e. by the presence of virus in the excretions of an infected bird, as recently announced by Hammar *et al.*[6] In any event, the epidemiology and the extent of the animal and bird reservoir of eastern equine encephalitis has not adequately been defined. It has been well established, however, that pheasants are particularly vulnerable and that the infection, when acquired by this species, gives rise to serious lesions and a high mortality. During the period from 1938 to 1946, 13 epizootics occurred among pheasants in New Jersey as reported by Beaudette and Black.[1] From brain tissue of the sick birds eastern equine encephalitis virus has been repeatedly isolated by these workers, particularly in recent years.[2]

Within the State of Connecticut, epizootics of encephalomyelitis have also occurred not infrequently among ringnecked pheasants which are raised for hunting purposes on local farms. The last widespread outbreaks occurred in 1950 and 1951.[7] From these sick pheasants eastern equine encephalitis virus has also been isolated.[8] One of the important epidemiological observations made in this connection has been of cases within single

pens of pheasants without spread to flocks which were in adjacent pens and separated only by a chicken wire fence. There was no comparable disease reported simultaneously among the human population or among horses in the same localities.

Such a situation has led to a number of enquiries as to how the pheasant disease was being transmitted from bird to bird, and how the absence of human or equine cases could be explained in view of the abundance of sick birds. Obviously this has raised the questions as to whether the absence of human cases was due to lack of transmission of the virus from pheasant to human or to the presence of inapparent human infection, leading to immunity in human beings.

In seeking an answer to the above questions, it seemed logical that the local pheasant farmers and handlers should be studied first. If there were transmissions of the infection from pheasant to man, specific antibodies ought to be present in appreciable quantities in the serum of the people engaged in the trade. With this in mind, a field team* was organized in December 1952 to collect sera from pheasant farmers, from persons who had contacts with horses which had died from this disease, and from convalescent patients who might have had this disease in 1938. Attempts were also made to isolate the virus from the sick pheasants.

MATERIALS AND METHODS

A. *Human sera.* All the human sera were separated within 24 hours after the bloods were collected, and then frozen and kept at —30° C. until immediately prior to the performance of neutralization tests.

1. *Sera from pheasant farmers and handlers.* During the period from December 19, 1952 to January 4, 1953, about 90 large pheasant farms were visited, including most of those where severe losses of the birds had been reported during the epizootic in 1950 and 1951, and also three large pheasant packing plants in Connecticut. The geographic distribution of the sera collected was shown in Figure 1.

A total of 215 samples of sera were obtained from as many individuals. All of these were from persons who had had, or were in, close association with pheasants. Attempts were made to include as many youngsters as possible because children were often admitted into the pheasant pens. The age distribution of the individuals from whom the blood samples were obtained is shown in Table 1. It ranged from 3 to 85 years of age, with almost one-quarter between 10 and 25 years and one-half between 30 and 55 years.

2. *Sera from individuals who had close association with horses dying of viral encephalitis.* On July 27, 1953, one horse died with typical symptoms and signs of

* The field team consisted of S. J. Liao, M.D., A. E. Burke, Ph.D., and Messrs. (now Drs.) R. F. Hustead and J. J. Nora, then third-year medical students at Yale University School of Medicine. The field work was supervised by Prof. John R. Paul, M.D.

encephalitis on a farm at Kenyon, R. I. Another horse on the same farm died on September 25, 1953, with a similar disease. From the second horse, EEE virus was isolated (from its brain stem but not from the cerebrum) using embryonated eggs, by Dr. Vance Yates of the University of Rhode Island. Through the generous help of Dr. Yates, one sample of blood was obtained on October 25, 1953, from each of the two persons who took care of the sick horses. These two individuals were aged 18 and 48 years respectively.

3. *Sera from individuals who were supposed to have had viral encephalitis in 1938.* One sample of blood was obtained from each of seven individuals through the generous

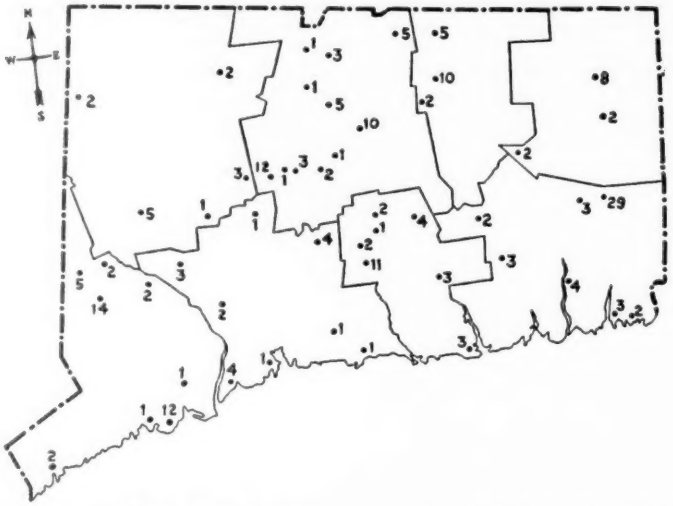

FIG. 1. Geographical distribution of serum samples collected in Connecticut in December 1952. Each dot indicates the site where one or more samples were collected; the number indicates how many samples were collected in each location.

help of Dr. R. E. Thalberg of Southington, Connecticut, and from one individual seen at the New Haven Hospital. In 1938, these persons had been seen in consultation by Dr. John R. Paul and the late Dr. James D. Trask.[10] All were thought to have had viral encephalitis (type undetermined) at that time, but no encephalitis virus* or antibody studies were successfully completed during their illness.

4. *Sera from laboratory personnel who were inoculated with EEE vaccine.*† Pre- and post-vaccination samples of serum were obtained from five persons. One of them had three subcutaneous injections of 0.5 ml. each, five months before the blood sample was obtained and had been exposed to the virus repeatedly during that period. Two

* Other than a search for poliomyelitis virus by monkey inoculation of fecal extracts.
† The vaccine was kindly supplied by Lt. Col. Robert Yager, V.C., Washington, D. C.

persons had two such injections, and another two had only one injection. The post-vaccination samples of these last four individuals were taken about 3½ months after the last injection. During that period of time, none of these four were exposed to the virus.

SEROLOGICAL TECHNIQUES

1. *Neutralization tests.* For screening purposes, two doses of the EEE virus were employed, viz., 10 and 100 LD_{50}. The human sera were neither diluted nor heated. Equal amounts of the serum and of the appropriate dose

TABLE 1. AGE DISTRIBUTION OF SURVEYED POPULATION

Ages	*No. of individuals*		*%*	
0-4	2		0.93	
5-9	6		2.78	
10-14	12		5.55	
15-19	25	51	11.57	23.60
20-24	14		6.48	
25-29	15		6.94	
30-34	18		8.34	
35-39	25		11.57	
40-44	34	120	15.74	55.56
45-49	23		10.65	
50-44	20		9.26	
55-59	6		2.78	
60-64	9		4.16	
65-69	2		0.93	
70-74	1		0.46	
75-79	2		0.93	
80-85	2		0.93	
Total	215		100.00	

of the virus were mixed and left in an ice bath in a refrigerator overnight. 0.02-0.03 ml. of the mixture was inoculated intracerebrally into each of five mice (3-5 weeks old). The animals were observed for 10 days. With each experiment, a complete titration of the virus and of the hyperimmune serum was incorporated.*

2. *Complement fixation test.* For screening purposes, the human sera were diluted 1:2. The sera were heated at 60° C. for 20 minutes before testing. The complement fixing (CF) antigen was kindly supplied by Dr.

* A quantity of the hyperimmune horse serum was generously given by Dr. Erwin Jungherr and Mr. Roy Luginbuhl, University of Connecticut, Storrs, Connecticut.

Joseph M. Smadel. Two exact units of complement were used in each test. The controls included normal mouse brain, 1 unit and 2 units of complement, the antigen and the hyperimmune horse serum. Only positive sera, found by screening test, were subsequently titrated.

NEUTRALIZATION RESULTS

I. *Sera from pheasant farmers:*

1. *Neutralization test against the prototype strain of the EEE virus.* Of all the 215 samples tested, only one sample had the highest neutralization index at 24; 8 (or 3.7%) had indices of between 10 and 25, and the remaining sera were all less than 10. Essentially, therefore, the tests were all negative insofar as indicating recent infection in these people.

2. *Neutralization test against the local pheasant strain of the EEE virus.** Only 45 samples of the 215 sera were tested with the pheasant strain. These included all those giving a neutralization index (to the prototype strain) of 10 or more and those giving a C-F titer of 1:2 or more. Only 12 of the 45 had neutralization indices to the local strain between 10 and 20. The result of individual serum obtained with these two strains of the virus were essentially the same, the differences being within the limits of the experimental error.

3. *Complement-fixation test with the prototype strain of the EEE virus.* Twenty-four sera of all the 215 samples showed titers between 1:2 and 1:4. There was no correlation between C-F titer and neutralization index of the same individual serum. The results were not considered significant.

II. *Sera from seven individuals thought to have had "viral encephalitis" 15 years ago.* Three of the seven persons had neutralizing indices between 10 and 20 against the pheasant strain of virus. The remaining four were below 10.

Only one of the seven had a C-F titer of 1:4, while the remaining six were negative at this level.

III. *Sera from two individuals exposed to horses dying of EEE.* Both had neutralization indices of less than 10, and C-F titers of less than 1:4.

IV. *Sera from the vaccinated individuals.* Neutralization indices were as follows:

* The isolation of this strain was described in a later section of this report.

Name	*No. of injections received*	*Neutralization index*	
		Pre-	*Post-immunization*
D.D.	1	10	125
I.M.	1	10	100
P.H.	2	10	90
S.L.	2	10	200
M.R.	3	not done	200

None of the five had any detectable C-F antibodies in both the pre- and post-immunization samples at the level of 1 : 4.

VIRUS ISOLATION FROM LOCAL PHEASANTS, 1953*

A single outbreak of encephalitis among pheasants on a farm in Farmington, Connecticut, was reported in the fall of 1953. There were no cases of encephalitis reported in human population or among horses in this state during that period. This epizootic started in late September and lasted about five weeks. Of 400 birds on this farm, 225 were sick with typical symptoms and signs of encephalitis during that time. Practically all sick birds during the early part of the outbreak died, while relatively more recovered during the later part. The sickness was confined to a single pen, and none of the birds in adjacent pens, separated only by chicken wire fence, became ill. On October 20 and 21, 1953, when the epizootic was approaching its end, brain tissues were obtained from five diseased pheasants with aseptic techniques and immediately frozen on dry ice.

Materials from two sick pheasants with encephalitis were also obtained on a farm near Kingston, Rhode Island, less than 10 miles away from the aforementioned farm in Kenyon, Rhode Island, where two horses died of virologically proven eastern equine encephalitis.†

1. *Technique.* Each pheasant brain which had been kept frozen, was ground aseptically and a 20% suspension was made with a M/10 phosphate buffer at pH 7.2 containing 0.5% crystalline bovine albumin. The suspensions were inoculated into a number of adult mice (3-5 weeks old) intracerebrally, and a number of baby mice (2-7 days old) intracerebrally and subcutaneously, or intraperitoneally. In addition, the same materials were

* Mr. Luginbuhl informed us that EEE virus was repeatedly isolated in his laboratory from the brains of sick pheasants on the same farm (where we obtained our material) during the first two weeks of October, 1953, by means of embryonated eggs.

† Through the kindness of Dr. Vance Yates.

also inoculated into the chorio-allantoic cavity of 9-10-day-old embryonated eggs. At the time when the isolation experiments were carried out, no other experiments in which EEE virus was used were in progress in the same laboratory.

2. *Results.* From only one of the seven birds was an agent isolated. This brain was from the Connecticut farm. The original brain suspension caused severe paralysis of limbs of two-day-old baby mice (4 out of 4) with an incubation period of 16 days by intracerebral and subcutaneous routes, and severe paralysis of one out of 15 adult mice inoculated by intracerebral route with an incubation of 14 days. The incubation period of the brain materials from these paralyzed mice was shortened to seven days on the first passage to adult mice and to three days on the second passage to adult mice. For example, the LD_{50} of the first passage material was 10^{-3} and that of the second passage was 10^{-5} when titrated in adult mice, and the LD_{50} of the second passage material was 10^{-7} in 2-5-day-old mice by the intraperitoneal route. When this particular original suspension of the pheasant brain was inoculated into embryonated eggs, no hemorrhage or death of the embryos was observed for ten days. The chorio-allantoic fluid of these inoculated eggs did not cause any sickness in adult mice for a duration of 30 days. It was not until the time when second passage mouse brains were used, that infection in 9-10-day-old embryonated eggs occurred, with hemorrhage and death of chick embryos in 40 hours. The agent was neutralized by the EEE hyperimmune serum, but not by West Nile or FA sera.

None of the other six pheasant brains caused any apparent disease in embryonated eggs or in mice. These isolation studies seemed to emphasize certain precautions in attempts to isolate EEE virus from sources which might have only a meager amount of the infectious agent.

COMMENT

This study yielded the answer to only one of several questions posed earlier in this report. This answer is that there was no serological evidence that inapparent infection with eastern equine encephalomyelitis virus had been occurring with any degree of frequency among pheasant farmers in Connecticut, some of whom presumably had had the opportunity for exposure to this infectious virus. The absence of appreciable amounts of neutralizing antibodies against EEE virus among the pheasant farmers is a significant negative finding. How this disease, which seems to be relatively common among local pheasants, is transmitted from bird to bird, and why it was not transmitted from bird to man in spite of their close association

during an epizootic are unanswered questions. In the opinion of local farmers in various parts of this state, there was no dearth of mosquitoes during those epizootic periods. If this is the case, and if mosquito vectors are of vital importance in the transmission of this disease, it seems likely that one or more "suitable" species of arthropod, which might serve as a vector for this virus, carrying it from bird to man or even bird to bird, has been absent in this part of the country during the past few years. This is indeed a matter requiring much more study.

Another feature which has come out of the work has been the failure to find neutralizing antibodies in the sera of those who were thought to have had "encephalitis" in 1938, presumably of eastern equine variety. The negative findings raise the question as to whether more than one virus might not have been present that year and that the Connecticut cases of "encephalitis" might have been due to some virus other than eastern equine encephalomyelitis as a result of an influx of new and potent arthropod vectors rather than an influx of one virus. This is a point which also deserves further study.

SUMMARY

A serological survey of pheasant farmers in the State of Connecticut failed to reveal appreciable levels of EEE antibodies in those individuals who had been presumably exposed during an epizootic among the pheasants about a year previously.

Included in this survey were also sera obtained from seven persons who had suffered from "viral encephalitis" in 1938. None of these had any significant amounts of the EEE neutralizing or CF antibodies.

It is believed that both of the above findings represent significant negative findings.

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A STUDY OF CALCIUM AND MAGNESIUM IN THE CEREBROSPINAL FLUID†

In the course of studies conducted in this laboratory on the distribution and concentration of metal ions in the central nervous system (CNS),[15] similar studies were made on the cerebrospinal fluid (CSF). Of the fifteen ions found in the CNS tissue, only four (Na, K, Mg, and Ca) were found consistently, and Cu occasionally in CSF. The rôle of magnesium in biological systems has been well studied.[10, 12, 19] The rôle of calcium in the same systems is not so well understood. In general, it would appear that calcium and magnesium are antagonistic to each other in their action on enzyme systems. The mode and site of action of these antagonists is not clear but may involve the shifting of equilibria through activation or inhibition of different mutually dependent components of the same system.[1, 6, 11, 13, 16, 17, 20, 24, 28] There is also evidence that these ions may be competitive. Magnesium, the concentration of which is very important in cells where high metabolic activity or high energy production-requirement exists, has been shown to be required for the activation of most of the enzymes concerned with the transfer and removal of phosphate in biological systems.[14] The CNS has been shown to have such an energy requirement.[18, 22] It has further been shown that the CSF is not a filtrate but is more in the nature of a dialysate of the plasma, and, while the origin of this fluid and the location of the barriers interposed between it and the blood are still a matter of controversy, nevertheless, it is generally accepted that this fluid is more nearly in equilibrium with the cells of the CNS than is the plasma.[21, 26] It seemed worthwhile, therefore, to make a study of magnesium and calcium in the CSF which might elicit some information of the metabolic components of the CNS cells since the interstitial fluid in which they are bathed closely resembles the CSF.

A search of the literature revealed that the Clark-Collip method for the determination of calcium in the CSF was most widely used.[4] There was also

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† Part of the material in this paper was taken from a thesis submitted by Edmund Sonnenblick in partial fulfillment of the requirements for the degree of B.A. with Distinction, Wesleyan University, 1954.

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revealed a number of methods for magnesium. However, in spite of the fact that normal values had been established for both ions, in no case were the levels of both of these ions reported in the same fluid. It was felt that the levels of magnesium and calcium and their ratios in the CSF could be of significance. It was decided to adapt the ethylenediaminetetraacetate (EDTA) methods for calcium and magnesium determinations in body fluids to CSF.[2,3,7] While this work was in progress, Stutzman and Amatusio reported calcium and magnesium levels and their ratio in the CSF.[27]

EXPERIMENTAL

The method finally decided upon for the determination of calcium was a modification of the method of Buckley and Bartolotti.[3] The total magnesium and calcium was determined by using eriochromeschwarz-T indicator and titrating with standard EDTA. Thus, by subtracting calcium values from calcium-magnesium values, calcium and magnesium levels were easily and rapidly determined in a single specimen of the CSF. These methods were checked by the Clark-Collip method for calcium in body fluids and the method of Fales for magnesium.[8] In standard addition and recovery experiments of calcium and magnesium done on pooled samples of the CSF, the percentage deviation was less than 2%. In studies of the reproducibility of calcium and magnesium determinations, the percentage deviation was also less than 2%.

PROCEDURE

CALCIUM: One ml. aliquot of CSF was placed in a 50-ml. white porcelain evaporating dish; 5 ml. of distilled water, 0.15 ml. of 5-N sodium hydroxide, and 1 ml. of ammonium purpurate (murexide) were added. The solution was titrated with standardized EDTA from a salmon-pink to an orchid purple at the end-point.* Milligrams per cent calcium were calculated.

CALCIUM-MAGNESIUM: One ml. aliquot from the same CSF was placed in a clean Erlenmeyer flask; 10 ml. of buffer solution and one drop of erichrome-schwarz-T indicator were added. The mixture was titrated with standardized EDTA from a starting red color to a clear blue end-point. Results were calculated as magnesium. Since the total calcium and magnesium were determined by this titration, by subtracting the calcium determined previously from the total calcium-magnesium, calcium, and magnesium concentrations in the particular CSF were obtained.

* It was found that the delicate color change could not be read accurately in glassware. Contrary to other investigations, no difficulty was found in reading the end-point if a clean, white, porcelain dish was used and a bit of care was taken to notice the color contrasts produced by EDTA.The end-point is reached when no more color contrast exists. Reproducibility was obtained with a little practice.

REAGENTS

EDTA: Approximately four grams of EDTA was dissolved in a small amount of distilled water and diluted to one liter. One ml. of this is equivalent to approximately one mg. of calcium carbonate. The exact equivalence for calcium was determined by titrating with standard calcium carbonate. The exact equivalence for magnesium was determined by titrating with standard magnesium chloride. For determining calcium and magnesium in CSF, this solution is diluted one in forty with distilled water. One ml. of this reagent is then equivalent to one mg.% of calcium and 0.600 mg.% of magnesium.

AMMONIUM PURPURATE (murexide): 0.1 grams of the powdered indicator are dissolved in 50 ml. of distilled water and stored in a dark place. This solution should be made fresh every three days.

ERIOCHROMESCHWARTZ-T: 0.5 grams of the indicator are mixed with 50 ml. of triethanolamine and placed in a glass bottle. If this solution is kept slightly basic, it is quite stable and easily handled.

BUFFER SOLUTION: 67.5 grams of ammonium chloride are mixed with 570 ml. concentrated ammonium hydroxide and diluted to one liter.

SODIUM HYDROXIDE: A 5-N solution of sodium hydroxide is prepared.

RESULTS

Calcium and magnesium levels were determined on the CSF obtained from forty patients who met the following conditions:

a. No sign of neurological condition.

(b) No psychosis or neurosis.

The mean level for calcium was found to be 4.95 mg.% $\pm$ 0.11. The mean level for magnesium was 3.01 mg.% $\pm$ 0.06. The calculated $Ca^{++}/Mg.^{++}$ ratio was 1.64 $\pm$ 0.03 (Table 1).

In addition, calcium and magnesium were determined on the CSF and serum obtained at the same time from ten patients selected as above. As shown in Table 2 the CSF calcium averaged 4.97 mg.% $\pm$ 0.11 and magnesium 3.14 mg.% $\pm$ 0.25, with a ratio of 1.61 $\pm$ 0.07. Serum calcium averaged 9.97 mg.% $\pm$ 0.15 and magnesium 2.49 mg.% $\pm$ 0.18 with a ratio of 4.1 $\pm$ 0.7. The value of 4.26 mg.% CSF magnesium in patient No. 6 was the reason for the deviation of the CSF magnesium and $Ca^{++}/Mg.^{++}$ ratio in this series from that of the values obtained in the original series of forty patients.

DISCUSSION

The EDTA methods for the determination of magnesium and calcium in the CSF was found to be rapid, simple, and reliable. It has the added advantage of requiring only one ml. of fluid for the analysis of each ion. Previous investigations of the concentrations of calcium and magnesium

TABLE 1. CALCIUM AND MAGNESIUM IN THE CEREBROSPINAL FLUID OF PATIENTS SELECTED AS NORMALS

Patient No.	*Ca mg%*	*Mg mg%*	*Ca++/ Mg++*	*Patient No.*	*Ca mg%*	*Mg mg%*	*Ca++/ Mg++*
1	4.79	2.94	1.63	21	4.73	2.90	1.63
2	4.79	2.97	1.62	22	5.16	3.12	1.66
3	4.73	2.97	1.60	23	4.79	2.90	1.65
4	4.81	2.90	1.66	24	5.21	3.21	1.67
5	4.84	2.93	1.65	25	5.11	3.03	1.68
6	4.94	3.06	1.62	26	4.78	2.99	1.62
7	4.79	2.90	1.65	27	4.87	3.03	1.62
8	5.16	3.09	1.67	28	4.94	2.96	1.67
9	4.84	2.96	1.64	29	5.00	3.03	1.65
10	4.94	3.12	1.60	30	4.89	2.99	1.64
11	4.79	2.93	1.64	31	5.11	3.09	1.65
12	4.94	2.99	1.65	32	5.00	3.12	1.60
13	4.94	2.99	1.65	33	5.32	3.22	1.65
14	5.00	3.06	1.63	34	5.05	3.06	1.65
15	5.01	3.00	1.66	35	5.11	3.09	1.65
16	5.06	3.16	1.60	36	4.79	2.93	1.64
17	4.85	3.02	1.61	37	5.06	3.06	1.65
18	4.89	3.03	1.61	38	4.79	2.93	1.64
19	4.79	2.99	1.60	39	5.00	3.06	1.63
20	4.84	2.90	1.67	40	5.01	3.00	1.66
				Mean	4.95	3.01	1.64
				Av. Dev.	0.11	0.06	0.03

in the CSF have shown wide normal ranges. Thus, Merritt and Fremont-Smith[23] have reported a range of 4.5-5.5 mg.% for calcium with an average of 3.05 mg.%. Flexner[9] has reported a range of 4.0 to 7.0 mg.% for calcium and 3.04-3.65 mg.% for magnesium. Cohen[5] has reported 1.0-3.5 mg.% for magnesium. More recently Stutzman and Amatusio[27] have determined calcium and magnesium in the CSF using the Clark-Collip method for calcium and the Simonson hydroxyquinoline method for magnesium. These investigators reported 4.86 ± 0.13 mg.% for calcium and 2.88 ± 0.17 mg.% for magnesium. This compares very well with the results of the

present investigation where CSF calcium is found to be 4.95 ± 0.11 mg.% and magnesium 3.01 ± 0.06 mg.% (see Table 1). It seems fairly certain that the normal range of calcium and magnesium in the CSF is maintained with very narrow limits.

Another interesting finding is apparent from this work for there is a remarkable constancy in the $Ca^{++}/Mg.^{++}$ ratio as shown in our forty cases selected as normals. The ratio of 1.64 ± 0.03 (see Table 1) agrees very well with the value of 1.66 ± 0.06 found by Stutzman and Amatusio.

TABLE 2. SERUM AND CEREBROSPINAL FLUID CALCIUM AND MAGNESIUM FROM A SELECTED GROUP OF RELATIVELY NORMAL PATIENTS

No. Patient	*CSF* *mg% Ca*	*mg% Mg*	*Mg++ Ca++/*	*Serum* *mg% Ca*	*mg% Mg*	*Mg++ Ca++/*
1	4.89	2.96	1.65	9.89	2.45	4.1
2	4.89	2.93	1.67	9.80	2.39	4.1
3	5.00	3.03	1.65	10.09	2.23	4.9
4	4.84	2.96	1.63	9.83	2.26	4.3
5	5.05	3.00	1.68	9.95	2.32	4.3
6	5.16	4.26	1.21	9.95	2.87	3.5
7	4.94	2.96	1.66	10.00	2.19	4.6
8	5.26	3.26	1.62	9.80	2.53	3.8
9	5.06	3.06	1.65	10.15	2.51	4.0
10	4.79	2.93	1.64	10.20	2.33	4.3
Mean	4.97	3.14	1.61	9.97	2.49	4.1
Av. Dev.	0.11	0.25	0.07	0.15	0.18	0.7

This value holds even though the absolute amounts of calcium and magnesium may vary. In view of the fact that calcium and magnesium are mutually antagonistic, and that magnesium is associated with high metabolic activity and high energy production systems, it is suggested that the CNS requirement for these ions is different from other body systems and that the ratio of these ions is rig ly controlled by some highly selective mechanism under normal conditions. The ten cases where the CSF and the serum Ca^{++}/Mg^{++} ratio were determined supports this assumption since the serum ratio was at 4.1 as against 1.65 for the CSF. Investigations are now under way to determine serum and CSF calcium and magnesium and their ratios in patients with varying degrees of mental disturbance. Deviations such as occurred in patient No. 6 (Table 2) may be significant since preliminary work suggests that there is a distinct change in the $Ca^{++}/Mg.^{++}$ ratio of disturbed patients.

ACKNOWLEDGMENT

We wish to thank the following members of the staff of the Connecticut State Hospital, Middletown: Jorge Paras, M.D., who secured most of the spinal fluids for us; Nina Toll, M.D., who furnished us with CSF from selected mentally disturbed patients for preliminary exploration; and Joseph A. Beauchemin, M.D., Director of the Clinical and Neuropathology Laboratory, for his advice and support throughout this work.

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ZOOLOGICAL JOURNAL CLUB

December 15, 1954

POPULATION DYNAMICS OF THE SARATOGA SPITTLEBUG, *Aphrophora Saratogensis,* IN THE LAKE STATES. By D. M. Benjamin, University of Wisconsin.

Upwards of 50,000 acres of hard pine plantation have had to be treated to prevent destruction by the spittlebug, an insect with a distribution from Novia Scotia to Florida and California. Although reported since 1848, it has become a menace only since 1948 and then only in certain regions. The damage to the trees is due to the withdrawal of sap through the insects' feeding punctures and a fungus infection that follows puncturing, the affected branches and twigs dying a year later. Provided the density of punctures per branch is not too great, the tree may recover upon the removal of the insect. DDT is effective for this.

The spittlebug lays its eggs in August in the terminal shoots of twigs. Hatching occurs the following spring, the larvae then depending on an alternate woody-plant host which is unaffected by the larvae's presence. In June, transformation to the adult stage occurs. Thus, population density is dependent upon a critical density of the intermediate host species. When these are insufficient, not enough adults can hatch to seriously damage the pines.

The rather specific changes in the growth patterns of pines which are the result of spittlebug infestation enable quantitative estimates of the insect population density to be made.

P. K.

YALE MEDICAL SOCIETY

January 10, 1955

ACQUIRED RESISTANCE TO VIRUS TOXIC ACTION. By Robert R. Wagner, Department of Internal Medicine.

Acquired resistance to toxic action of several strains of influenza, Newcastle disease, and mumps viruses can be demonstrated using body temperature and circulation lymphocyte counts as the chief indicator systems. Experiments in conjunction with Dr. I. L. Bennett, Jr. have shown marked rises in rectal temperature in rabbits following I.V. injection of large doses of virus. Harris and Henle previously described a fall of up to 80% in the total number of circulating lymphocytes following similar injection. These reactions are caused by the elementary body since neither pyrexia nor lymphopenia can be demonstrated following high-speed centrifugation, erythrocyte absorption, or neutralization with homologous antiserum. Heating sufficient to destroy infectivity, but below the critical temperature that destroys virus hemagglutination, does not prevent the toxic reaction indicating this property to be distinct from infectivity. Compared with reaction to bacterial pyrogens the latent period of virus-induced fever is at least six times longer and the temperature elevation slightly lower.

Relative resistance to bacterial pyrogens can be produced by repeated daily injections of endotoxin. Virus resistance is more quickly acquired. Following a single injection of 1 ml. of the Lee strain of Influenza B, a challenge dose of homologous virus 24 hours later produced almost no pyrexia or lymphopenia. A similar refractory state can be demonstrated with Newcastle disease, mumps, and influenza A viruses. Furthermore, animals resistant to any one strain are also resistant to the others indicating that tolerance to pyrogenic and lymphopenia-producing action of these viruses is not strain specific.

Extension of this work to mice, using inhibition of neurotoxic effect as the indicator system, shows that this virus property is not limited to the rabbit temperature-regulating mechanism. Resistance to neurotoxicity can be demonstrated before production of antibodies and, indeed, diminishes as serum antibody titer increases, demonstrating that resistance to virus toxic action is different from specific immunity.

RICHARD I. BREUER

GONADOTROPHIN TITERS IN DISTURBED PREGNANCIES. By John McLean Morris and Edward Hon, Department of Obstetrics and Gynecology.

The levels of urinary chorionic gonadotrophin excretion during normal pregnancy follow a characteristic curve. If the highest and lowest values compatible with a normal pregnancy of a given duration are known, and a titer is obtained that is outside these limits, it should be possible to state that a serious disturbance in pregnancy exists and that the patient will not produce a viable infant. Since chorionic gonadotrophin levels are high in early pregnancy when the majority of these disturbances occur, it should be possible to select with an adequate margin a level beneath which titers are considered pathologically low. This theoretical level is indicated at 3000 *i.u.* per 24 hours from days 50-90 after the last normal period.

The toad, *Bufo americanus,* is used as the assay animal. Careful extensive determinations of the sensitivity and seasonal variability in sensitivity of the toad to standardized chorionic gonadotrophin considerably increases the accuracy of the assay method. Also, a urinary concentration technique (described in Yale Journal of Medicine and Biology, December, 1954) has eliminated the problem of false negatives. In 652 routine pregnancy tests there were no false positives or false negatives in determinations made provided more than 10 days had elapsed since the last missed period.

A study of 122 cases of disturbed pregnancies showed that all patients having a chorionic gonadotrophin value of less than 3000 *i.u.*/24 hours from day 50 to day 90 went on to abortion. In this study, the toad method proved accurate in 83 cases in predicting abortion, whereas in 35 of these 83 cases, the A-Z test, which was run as a monitoring test, did not suggest that the pregnancy would terminate in abortion.

The method of the toad test consists of injecting urine into the dorsal lymph sac; if present in sufficient titer, the gonadotrophin stimulates the release of sperm within one to four hours which are withdrawn and demonstrated by microscopic examination.

All observations thus far indicate that the toad assay method is a useful laboratory test enabling one to distinguish threatened from inevitable abortion, indicating when conservative measures should be employed or when immediate surgery should replace protracted periods of bed rest and medication. Experience proves this test to be a practical, accurate, rapid, and inexpensive test for diagnosis and prognosis in problems associated with gonadotrophin titers and suspected disturbances of pregnancy.

MYRON M. LOTZ

HENRY BUNTING MEMORIAL LECTURE

January 20, 1955

CHROMOSOMES: THEIR CONSTITUTION AND FUNCTION. By A. R. T. Denues, The Sloan-Kettering Institute for Cancer Research, New York, New York.

By means of the phase-contrast optical system combined with electron micrograph studies, it has become possible to study the living and fixed nucleus in greater detail than ever before. Our present concept of the nucleus is as a cellular organelle with a duality of function. One of these is the necessity of the nucleus for cell heredity. The constancy of its desoxyribose nucleic acid (DNA) fraction from cell generation to cell generation and the changes in DNA content in certain stages of cell division are proof of this. Also the fact that enucleated cells do not divide although they may live for several weeks shows this important relationship.

The second rôle of the nucleus is in development. The work on Acetabularia shows that there is an interaction between the nucleus and the cytoplasm in differentiation; studies of the nuclear membrane indicate that it is permeable to large molecules (as large as hemoglobin). Electron micrographs show that the positively charged nuclear membrane (as opposed to the negatively charged nucleolus and chromatin) has what appear to be holes in its surface, and there is evidence in some photomicrographs of a transfer of material from nucleus to cytoplasm.

Chemical analyses of the nucleus have indicated that both basic and acidic proteins in addition to lipids and metals can be found in the nuclear sap. The most important and characteristic constituent of the nucleus is, however, DNA (even the nucleolus contains Feulgen positive material). Our present knowledge of this compound is best exemplified by the Watson-Crick model of the structure of the molecule, a core of purines and pyrimidines holding together a helical structure of extreme complexity, with the phosphates attached to the outside rather than in the core.

The chromosomes, which are the most dramatic visible structures within the nucleus, have been studied by means of both phase-contrast and electron microscopy. Studies of isolated chromosomes show structure exceedingly well; major and minor coils, as well as relational coiling, can be seen. The chromosomes of chick, rat, and man and also polytene chromosomes of Diptera have been studied in these ways.

Recent studies of spindle fibers show them to be real structures and not artifacts as some investigators have been led to believe. Phase-contrast and polarized light microscopy have given some insight into the rôle of the very complex spindle structure in cell division, although the problem is still far from being solved.

RALPH HILLMAN

PLANT SCIENCE JOURNAL CLUB

Febraury 1, 1955

THE ORIGIN OF LIFE. By G. E. Hutchinson, W. Vishniac, and E. C. Pollard, Departments of Zoology, Microbiology, and Biophysics.

The hypothesis that the Earth's original atmosphere consisted of methane, ammonia, and water vapor is probably quite reasonable. Miller and Urey have shown that when such a mixture is subjected to electrical discharges, amino acids will be formed, so such an atmosphere, with ultraviolet radiations in place of electrons, could provide for the origin of the building blocks of life.

The presence of vast amounts of CO_2 in a reduced state, the atmosphere and in limestone (12 to 21 atmospheres of it), poses a grave difficulty to Miller's hypothesis. Urey suggests that the water vapor of the early atmosphere was photolysized to produce O_2 and H_2, the latter escaping to outer space, the former oxidizing the methane to produce CO_2. Some intermediate accommodation of CO_2 before its deposition as carbonates still needs to be shown.

It is inconceivable that such a quantity of CO_2 as 12 atmospheres could be simultaneously present. It must have been added gradually, which is very likely true of other constituents, such as water. We would then expect that life began in rather shallow oceans, mere puddles really.

Meteorological phenomena have generally been overlooked, though they doubtless occurred early in the Earth's history the same as now. Rain could dissolve unstable reaction products, which, upon reaching the Earth's oceans, could sink to levels where the absence of ultraviolet radiation would grant them stability. This would greatly increase the variety of substances that could be formed. (Hutchinson)

It has been shown that the ratio of S^{32}/S^{34} remained constant over a long period during the Precambrian period. The increase in the proportion of S^{32} some eight hundred million years ago gives evidence of a turnover which was probably due to sulfur bacteria. Today, we know of sulfate-reducing, anaerobic bacteria which occur in tremendous numbers. The question of what preceded these organisms remains, however.

The simplest organisms of today, those that can live on CO_2, are the most complex biochemically, for the utilization of simple compounds requires the presence of complex mechanisms, of templates, or primers. Thus, a heterotrophic organism was probably the more primitive form. (Vishniac)

A great amount of faith is involved in accepting Miller's experiment as showing how life may have originated. Information theory (the engineers'

only intellectual contribution to science) tells us that 10^{12} questions have to be answered for a bacterial cell to be put together. The probability of random assortment forming such a cell is of the order of the age of the universe raised to its own power—which considerably weakens the faith in the origin of life à la Miller.

Cells which may synthesize proteins or amino acids or combine these with DNA to produce functional wholes cannot synthesize themselves. Such processes always depend upon the prior existence of the same material which is synthesized. The fact that amino acids could have been produced in a manner compatible with geochemical evidence, that physical laws are adequate for cellular processes, and the dictum by famous physicists that the problem of the origin of life is a worthwhile one justifies a spark of faith; 10^4 intermediate steps, however, still remain. (Pollard).

P. K.

BOOK REVIEWS

THE BACTERIAL FACTOR IN TRAUMATIC SHOCK. By Jacob Fine. Springfield, Ill., Charles C Thomas, 1954. 82 pp. $2.75.

This short monograph presents the point of view of a group of investigators at Harvard who have been conducting animal experiments on the problem of shock during the past decade. The initial chapters review and reject various theories which have been offered to explain the phenomenon of irreversible shock, such as increased capillary permeability, fluid and electrolyte imbalance, functional failure of vital organs, or liberation of endogenous toxins. The remaining chapters are concerned with results of animal experiments.

Traumatic shock was induced in dogs, either by blood-letting or by prolonged application of tourniquets. It was found that a significant proportion of the animals developed fulminating infections, and that these were the real cause of the state of irreversible shock. The suggestion is made that secondary bacterial infection may be of similar importance in irreversible shock in man. Certainly it must be admitted that patients with overwhelming infection can present the clinical picture of shock. However, since normal human tissues (unlike those of the dog) rarely contain viable bacteria, the point to be established is the frequency with which fulminating infection develops as a complication of traumatic shock in man. The work is interesting, readable, and provocative.

PAUL B. BEESON

PLAGUE. By R. Pollitzer. World Health Organization Monograph Series, No. 22. Geneva, World Health Organization, 1954. 698 pp. $10.00.

Plague has probably been the greatest threat to the human race not devised by man himself. The epidemic of the time of Justinian (542-600) is estimated to have had one hundred million victims; it "depopulated towns, turned the country into a desert, and made the habitations of man to become the haunts of wild beasts." In the European pandemic of the 14th century half to two-thirds of the population of England is believed to have been killed by the Black Death, and the previous estimate that 25 per cent of the peoples of Europe died during the pandemic has lately been criticized as an underestimate (data from Pollitzer). The work of medical microbiologists, particularly in the last two decades, has at last brought this, the worst of the man killers, under control, and now, wherever the skill and funds are available, it can be relegated to the status of a minor nuisance.

This classical subject has been given comprehensive treatment by a specialist who has had many years of plague experience. It is a pleasure to report that this book is written with the many-facetted awareness of the veteran worker but at the same time is completely up to date in the modern information offered and in its survey of current plague conditions throughout the world.

Treatment is systematic with chapters on History and Distribution, the Bacillus, Immunology, Pathology, Laboratory Diagnosis, Hosts, Insect Vectors, Clinical Aspects, Epidemiology and Control and Prevention. Two very useful appendices, (1) A List of Reservoirs and Vectors of Plague, and (2) Identification of Fleas (by F. G. A. M. Smit of the British Museum), conclude the volume.

Pollitzer does not accept the view that plague was introduced into North America (by Asiatics who carried plague with them) in the original colonization of the continent by way of the Bering Straits. It is difficult to see how the matter can be resolved with present means, but the argument advanced —that Asiastic and American strains differ biologically today so that they could not have had a common origin thousands of years ago—implies a belief in the stability of bacterial types which scarcely seems warranted.

The author discusses vaccination with live avirulent vaccines and bacterial products without indicating that a clear preference is justified. His comments on the rat population of cities are instructive. In well-sanitated cities the rat population is approximately one for every five inhabitants, whereas in the poorly sanitated settlements the rats exceed the human population.

Comments on therapy have an abrupt practicality which jolts. While the antibiotics are extremely effective, and with them even pneumonic plague patients almost always recover, antibiotics are not to be used routinely in many places. The cost differential favors the less effective sulfonamides, with the antibiotics being reserved for special or unresponsive cases.

Although the present situation in regard to plague is favorable, the author warns that the future will not necessarily be as good since we may be in the trough of an epidemic cycle and to some extent attributing to voluntary measures what is actually due to the ill-defined factors influencing plague periodicity.

The reading public in indebted to Dr. Pollitzer for this critical and extremely well-documented work. Althou.gh specialists may disagree on minor points they will probably find the book indispensible. It seems destined to become the standard work on the subject and to replace, for all modern plague topics, the treatise of Wu Lien-teh, which is now almost thirty years old.

DAVID WEINMAN

MEDICAL USES OF CORTISONE, INCLUDING HYDROCORTISONE AND CORTICOTROPIN. Francis D. W. Lukens, Ed. New York, the Blakiston Company, Inc., 1954. 534 pp. $5.00.

Basic knowledge of adrenal function and the clinical application of this knowledge have progressed so rapidly that very few physicians can keep up to date. The adrenal hormones have been used in the treatment of diseases of almost every body system, so that the modern physician must understand their use whatever his major field of interest. This volume attempts to summarize the scientific and clinical information about the adrenal cortex for the edification of the student who would otherwise be unable to collect and correlate the great mass of material now available.

The book contains fifteen essays, ranging from the Physiology of the Adrenal Cortex by Russell and Wilhelmi, through the Pharmacologic Aspects by Thorn and collaborators, to the various diseases (rheumatoid arthritis by Hench; rheumatic fever by Bunim; collagen diseases by Baehr; asthma by Rose, etc.). In each case the essay is written by an acknowledged

leader in the field, and each essay is complete and self-contained. The bibliographies are massive. Each author has had full freedom to express his personal opinions, so that the clinical portion of the book contains a number of contradictory statements, an expression of the present lack of understanding of the basic mechanisms by which the hormones work.

The very objectives of the book place it under some handicaps. The field has progressed so rapidly that certain aspects of the discussion were outdated before they were set in type; for example, the sodium-retaining potency of the amorphous fraction is now believed to be a result largely of aldosterone which is not mentioned in the volume. The fact that the book is not entirely up to date does not detract from its value; on the contrary, it emphasizes the need for a solid foundation on which the interested physician can build his newly acquired knowledge as it becomes available.

The fact that each essay is self-contained makes a considerable amount of repetition necessary. Whereas this repetition improves the reading qualities of each chapter separately, it tends to become a little monotonous when the book is read as a whole.

These minor criticisms should not detract from the basic value of the volume. It will long prove a useful guidepost for the practical application of the adrenal steroids as therapeutic agents.

PHILIP K. BONDY

VITAMINS IN THEORY AND PRACTICE. Leslie J. Harris, New York and London, Cambridge University Press. 1954. 4th ed. xxii, 352 pp. 103 illustrations; 105 tables.

This is the fourth and very much enlarged revision of a small volume that had its origin in a series of lectures by the author at the Royal Institution, London, some twenty years ago. It is not a textbook, but a popular presentation in a somewhat conversational and very readable style, without recourse to the irritating, racy language of the professional popular writer. It is beyond doubt one of the best and sanest accounts of the vitamin story for the intelligent reader that has been written, and is an excellent introduction to an understanding of the place of the vitamins in nutritional science.

The book will make an appeal particularly to the student of science and to the clinician—readers who are not alarmed by chemical formulas, but who wish a broad understanding of how we have arrived at our present knowledge of vitamins. It includes numerous figures and tables for those who think in terms of curves and diagrams, with a series of portraits including that of David Livingston, who reported xerophthalmia in Africa. The aim of the author is "to include in language intelligible to the lay reader some mention at least of every known fact of importance about the vitamins."

This volume is definitely historical in outlook and goes back to Lind (1757) and Captain Cook (1772). The later developments, beginning with Lunin (1888) through to the recent identification of the anti-anemia vitamins folic acid and vitamin B_{12}, are given in greater detail. Present-day accounts often give the impression that the vitamins were the result of chance discoveries or were merely the result of systematic laboratory feeding experiments which unearthed successively vitamins A, B, C, etc.,

enough to compose the present vitamin alphabet. That the vitamin concept, on the other hand, was evolved through a period of painful confusion and uncertainty is faithfully stressed in this volume.

The discussion of the best known vitamins A, B^1, C, D, E, and K is attractively written and that of vitamin C, on which the author is a recognized authority, is especially well handled. The author uses the terms "vitamin B^1," "vitamin C," etc. instead of the now generally used chemical names thiamin (aneurin), ascorbic acid, etc. The word "ferment" is used in discussing enzymes, a historical hold-over which might well be abandoned. No references are given throughout the book. There is no suggestion as to where the interested reader might turn for additional information.

In a volume whose perspective is essentially historical, mention of outmoded methods may be permissible. How otherwise justify space given to the bradycardia method for vitamin B^1 assay? One wishes that it had been possible instead to include an account of the recent vitamin B^1 studies at Bataan in the Philippine Islands. Many of the important vitamin studies in America, for example those of Sherman at Columbia and those from Mendel's laboratory here at Yale, receive scant mention.

A final chapter of fifty pages, entitled "Dietetics—What to Eat," is keyed to the nutrition situation in the United Kingdom and is only partly concerned with vitamins. This appendage fits a bit awkwardly on to the end of a book which is essentially an historical account of vitamins. It might more effectively be separated from the main volume and appear as a separate brochure.

WM. H. ADOLPH

THE DEAF AND THEIR PROBLEMS. By Kenneth W. Hodgson. New York, Philosophical Library, 1954. xx, 364 pp. $6.

The problem of severe deafness is nearly three times as frequent as that of blindness. If it comes after formal schooling is complete, when reading and speaking have been learned, and one knows a job and understands the world around him, it is not as great a handicap as blindness. On the other hand, deafness which sets in during childhood or infancy presents a tremendous problem in education. The young deaf child who cannot talk, cannot understand a word that is said, or read a word of anything that is written, is destined to a slow, laborious, costly, and frustrating process of learning.

The two main ways of teaching, in use since the work began, are: (1) the oral method by which children are taught artificially to speak words they cannot hear and also to lipread what is said to them; and (2) the silent methods depending upon the hands and utilizing either the manual alphabet, manual signs, or a combination. Because it is little taught, most "signing" today is unsystematic and degenerate, a "pidgin" language.

While the progress of science in the past few hundred years has revolutionized the attitude toward what Dr. Samuel Johnson called "the most desperate of human calamities," the attitude prior to the seventeenth century was one of fatalism and misunderstanding. Aristotle wrote, "Men that are born deaf are in all cases dumb," and "Those who are born deaf all

become senseless and incapable of reason." Aristotle's implication that the deaf could not possibly be taught to speak rendered them a tremendous disservice, since the next two thousand years of Christendom were to "live and die in Aristotle's works," and no educated man would waste his time and strength in essaying an acknowledged impossibility. The whole future of the deaf depended on the advance of knowledge.

Mr. Hodgson begins with a clear and concise description of the anatomy and physiology of the ear, and of the methods for testing its function in health and disease. From the sixteenth to twentieth centuries, beginning with the work of Bartolomeo Eustachi and Gabriele Fallopio, the author traces the developments in medicine and physics which enabled both physicians and educators to help their deaf charges.

American trends in the education of the deaf were influenced by two men, Thomas Gallaudet of Hartford, and Alexander Graham Bell, an English immigrant. Gallaudet, after working with l'Abbé Sicard in Paris, brought back with him the latter's manual method, as well as Laurent Clerc, Sicard's ablest pupil. Alexander Graham Bell stimulated interest in the oral method, using his father's system of "visible speech" to teach the deaf in his school at Boston.

Education of deaf children continues to be their chief problem. Because the parents of deaf children cannot communicate with them through the wall of silence, the author proposes substitute homes for the children, where they can live in little family groups in the care of married couples living an ordinary life, but trained in deaf teaching. The smallness of such home units would make them economically practical because they would be free of the tremendous overhead expenses of big institutions. The child would, in this way, enter school with good social adjustment and emotional response. In addition, a great deal would be known about him, his age level in language, his lip-reading ability, general educational attainment, character, temperament, etc.

The author concludes that the education of the deaf should be wholly the concern of the government. Such central responsibility alone can distribute the burden of the cost evenly over the whole community and give deaf children equal opportunities.

Mr. Hodgson's work presents the historical and philosophical aspects of the education of the deaf, then outlines the various legislative and organizational programs which have developed through the centuries to improve the social and economic status of "the deaf." Although presented from a British point of view, the material is international in scope, and provides a valuable background for anyone who is involved with the deaf in any locality or in any capacity.

JOHN A. KIRCHNER

BASIC ODOR RESEARCH CORRELATION. Roy W. Miner, Ed. New York, New York Academy of Science, 1954. Vol. 58 of the *Annals*, pp. 13-260. $3.50.

The book is divided as follows: Part I, The Odor Problem in Industry and Sciences, Part II, The Olfactory Process, Status of Present Knowledge,

Part III, The Status of Subjective and Objective Measurement Techniques; and Part IV, Experimental Studies in Odor.

Knowledge of the physical environment in which humans live is transmitted to the psychic centers through the special senses of vision, hearing, taste, smell, touch, and probably another sense which is frequently referred to as intuition or psychic perception or at times just the sixth sense. Of the first five, the senses of taste and smell have received the least amount of scientific attention and probably because without them the human being is least inconvenienced in his social and economic existence.

However, there are many interests within our country whose activities are partially governed by odors in the environment. The book herewith reviewed is a compendium of articles of some thirty authors.

The introduction is written by Albert R. Behnke and he outlines some of the research which has been done in the field of Olfaction. Some of the advances in human comfort because of odor control are mentioned and quotation is made from an article on Depth Electrography in which rhythmic potential discharges were recorded from an electrode in the region of the olfactory bulb.

The cigarette industry, the food industry, hotel management, and heating and ventilating engineers are all concerned with the odors to which their clients are subjected. Odors are modified, eliminated, or actually created to produce certain psychological effects. The huge perfume and cosmetic industry is directly dependent on the reaction of their customers to different fragrances, and it is well known that different odors can increase the pleasure of certain events in the life of a human being. Taste is so closely related to smell that it is often difficult to distinguish between the two, and one is made more pleasurable by the other at times, and at other times, less desirable. This is discussed from the viewpoints of a physiologist, a psychologist, a physicist, and a chemist.

Part II refers to the olfactory process itself and in six articles the status of personal knowledge is covered. Smell is indicated as a primitive sense which emerged long before vision and it is stated that even the simplest of animals such as the protozoa and coelenterates have some sort of chemical sensibility. In the higher animals the chemical sensibility differentiates a common chemical sense into taste and smell. The requirements for substances to be odorant are listed as, first, volatility, and second, solubility in the tissues of the olfactory region. The olfactory area and the olfactory receptor process are carefully described anatomically and historically.

The neural processes involved in odor perception are discussed at considerable length, based largely upon the work of E. D. Adrian. Further discussion of the neural mechanism of olfaction, the "olfactory brain," is the substance of an interesting chapter which includes an extensive bibliography. Olfaction in insects is also discussed and many references are made to previous work on this subject.

An information theory of olfaction is expounded with an introduction quoting Titus Lucretius Carus who developed the first atomistic theory of olfaction at about 47 B.C. Perhaps twenty-five theories of odor have been

advanced during the period of the last one hundred years. They center around the receptor and its relation with the odorant molecule. A classification of the theories is presented and the information approach to olfaction is discussed.

The measurement of odors and odor identification has a very practical application in industry and since there is yet no universal odor unit, measurement has been difficult. Specifications for ventilating installations, however, can use the dilution-threshold method.

Part IV takes up the experimental studies in odor which comprise a very small portion of the book. Only three articles are included, and it would seem from their perusal that further experimental work might interest those in the field of experimental psychology. Normal and neurotic monkeys lend themselves to observations of olfactory behavior and insects respond to a chemoreceptive process in controlled environments.

We are often aware of unpleasant odors, and nature has provided us with a large variety. Possibly we have not been aware of the basic odor research which has been going on, largely because the environment in which we find ourselves so frequently is not unpleasant for the reason that the odor engineers have anticipated our olfactory likes and dislikes.

NORTON CANFIELD

REPRODUCTIVE SYSTEM. The Ciba Collection of Medical Illustrations, Vol. II. Prepared by Frank Netter, edited by Ernst Oppenheimer. Summit, New Jersey, Ciba Pharmaceutical Products, Inc., 1954. xvi, 286 pp., 233 plates. $13.00.

This volume, second in the Ciba series "which will eventually portray all the major anatomy and pathology of the human organism," follows close upon the heels of its well-received predecessor *The Nervous System*. Heralded by a rhetorical foreword in which Dr. Rock, citing Malthus' predictions, suggests Man's ills stem in part from his reproductive exuberance, this work aptly fulfills its avowed objective to "supplement rather than replace the standard reference works. . . ." Consisting of 233 full-color, text-accompanied plates, 144 of which have appeared in previous Ciba Clinical Symposia, Volume II is divided into 14 areas dealing with the development, anatomy, pathology, and clinical evaluation of the male and female reproductive systems. While the excellence of Dr. Netter's illustrations has long been recognized by the medical profession, this collection further proclaims his ability to portray vividly human anatomy with almost uncanny 3-dimensional effect.

Though "offered as a nonprofit service to the medical profession and sold at the cost of publication and distribution," the price of thirteen dollars may considerably limit the market to which this book can successfully appeal. It is, nonetheless, to be highly recommended to both the practising physician and to the student in the clinical years.

J. C. C.

THE CONCEPT OF SCHIZOPHRENIA. W. F. McAuley. New York, Philosophical Library, 1954. 145 pp. $3.75.

The problem of schizophrenia, its etiology, pathology, and therapy, is one of the greatest challenges facing the medical investigator and practitioner today. As the author states, "A mental disorder that constitutes one-fifth of all admissions to mental hospitals and that accounts for 45 per cent of the resident population of mental institutions . . . is obviously a major problem of psychiatry."

Having established the necessity for active research in this field, the author undertakes to chronicle the major spheres of speculation and research connected with schizophrenia.

The first quarter of the book is devoted to an account of the evolution of the concept of schizophrenia as a disease entity by Kraepelin, Bleuler, Jung, Adler, and others in terms of intrinsic psychic dynamic mechanisms. Hypotheses concerning anatomical or physiological origins for the disease are discussed in detail, ranging from autonomic imbalance to pathologic reflex arcs. An amazing array of stimulating though often fanciful speculation is presented. Graphically demonstrated is the complexity and immensity of the problem for researchers.

Following this the author undertakes to define and study the major active spheres of investigation. The rôles of heredity, environmental and social conditions, neurophysiology and metabolism are presented, along with detailed accounts of representative experimental works in each of these areas of investigation.

The author presents the view that eventually the most accurate concept of this disease will be a fusion of a psychodynamic and organic approach. He emphasizes that studying the social relationships, family pattern, and early personality mechanisms is essential to an understanding of the origins and prevention of the disease. Essentially he defends the psychodynamic approach to schizophrenia and alludes to the contributions of psychoanalysis to an understanding of this problem. However, he does not succeed in presenting a coherent view of this problem which could be said to represent that of the present psychoanalytically derived dynamic psychiatry.

A brief essay on somatic therapeutic procedures, insulin coma, and leucotomy, omits discussion of stimulating recent advances and explorations in the field of psychoanalytic treatment of this illness, and in fact omits mention of any psychological treatment as such.

This work, then, presents the profusion of thought, speculation, and experimentation regarding the problem of schizophrenia. It succeeds in imparting the sense of immensity which this problem presents. However, rather than finding the concept of schizophrenia as the title implies, we become aware of the difficulty to be met in formulating such a concept. This book, then, is recommended only as a beginning of a survey of the scene, and not as a definitive work on the subject. The bibliography appears adequate, if not ample.

FREDERICK M. LANE

METABOLIC INTERRELATIONS. Transactions of the Fifth Conference sponsored by the Josiah Macy, Jr., Foundation, January 5 and 6, 1953. Edward C. Reifenstein, Jr., Ed. New York, Josiah Macy, Jr. Foundation, 386 pp. $5.00.

This is a report of one more Conference, the favorite indoor sport of the medical sciences. In the last paragraph of his introduction, before turning the meeting over to the chairman, the master of ceremonies, Frank Fremont-Smith, says: "Logic is a vital aspect of science, but equally essential is the intuitive or creative aspect. . . . By preserving the informality of our conferences in the published transactions, we hope to portray more accurately how the minds of scientists work and to give a true picture of the rôle which creativity plays in scientific research." Noble as this aspiration may be, it was predestined to failure. The *dramatis personnae* consisted of 26 experts with as many special interests and varying degrees of skill, experience, and information, of whom 24 were cast in important rôles. This necessitated some kind of formal program. Since, however, all the other participants were permitted, at any moment, to interrupt the speaker who had the floor, the threads of the arguments frequently became confused or utterly lost despite the best efforts of the stage-managing chairman. The cast was too small to warrant a formal program, but altogether too large for informal conversation. The characters of only a few of the most loquacious were revealed to the reading audience. A large proportion of the *ad lib* questions and remarks were not expressions of profound thought, but dealt with matters of technique. "*Sobel:* How much vitamin D did you give? *Copp:* There were 8 units per gram of diet, . . . about 50 units daily. *Butler:* That is a big dose. *Sobel:* It is a small dose. *Harrison:* No, it is a large dose. *Copp:* It is a large dose. *Follis:* Eight thousand units per kilogram of diet. *Copp:* Yes; 8,000 units per kilogram, that is right. It contains 8 units per gram; that is a better way of putting it" (p. 149). Such a passage proves only that the members of the Conference differed either in their facility at mental arithmetic at the decimal level or in their standards of magnitude. The "bigs" seem to have won by acclamation or vociferation. Such passages may have lent color to the scene for the spectators; for the reader they serve only to distract attention from the subject. The editor of this Conference seems to have lost his red pencil. The reader is even informed by the term "[Laughter]" what stirs the risibilities of distinguished physiologists. Sophisticated readers who really desire information will find it necessary to turn to the original articles of which the Conference report is a rehash.

JOHN P. PETERS

SHOCK AND CIRCULATORY HOMEOSTASIS. Harold D. Green, Ed. Third Conference on Shock and Circulatory Homeostasis of the Josiah Macy, Jr. Foundation, September 1953. New York, The Josiah Macy, Jr. Foundation, 1954. $3.50.

This record of the Third Conference on Shock and Circulatory Homeostasis of the Josiah Macy, Jr. Foundation, held in September 1953, resem-

bles the report of the previous conference in containing detailed experimental data and free roundtable discussions by an imposing roster of expert participants. Three chief topics were considered:

1. Dr. John M. Howard recounts his *experiences with shock in the Korean theater,* including the interesting observation of hypertension following milder trauma. The discussion is concerned with limitations of methods applicable to battleground conditions, with the effects of anesthesia and of blood replacement, and with an effort to relate clinical terminology and concepts to prior physiologic data concerning shock.

2. *Reflex factors in the regulation of the circulation* is concerned with the experimental work of Dr. G. S. Dawes on the reflexes and receptors regulating cardiac action and the blood pressure. The discussion provides a detailed critique of the sino-aortic mechanisms, as well as of the less well appreciated Von Bezold and pulmonary depressor reflexes.

3. *Functional properties of blood vessels* covers the interesting data of Robert S. Alexander concerning pressure-volume relationships in the aorta, intestinal circulatory loop, and isolated hind leg. Delayed compliance (stress relaxation, "hysteresis"), of blood vessels, the effects of CO_2 and anoxia on the systemic and pulmonary circulations, and the autonomous control of local circulatory beds are several of the more interesting problems discussed.

This volume is, like its two predecessors, of special interest to students of normal and abnormal cardiovascular physiology.

ALLAN V. N. GOODYER

SCIENCE AND CIVILIZATION IN CHINA, VOL. 1. By Joseph Needham. New York, Cambridge University Press, 1954. xxxviii, 318 pp. $10.

In 1949 this reviewer attended a seminar in the social history of science in which an attempt was made to delineate the conditions necessary for the flourishing of scientific activity. One of the areas studied was China and the problem was stated roughly, "Why did China never develop a science?" An expert in Chinese history was called in to help us bridge the enormous language barrier but even this expert never challenged the very assumptions of the problem as it was stated. It was just assumed that China had never had any science and our task was to discover, if possible, the reasons why.

It is this kind of assumption (all too common, one suspects, in these Western lands) that Professor Needham has set out to abolish in his monumental work. A glance at the table of contents for the four volumes which are already written (although only volume one has been published) and the tentative section headings of the three further volumes is enough to shatter, once and for all, the illusion that science is a characteristic peculiar to so-called Western Civilization. Here one finds mathematics, physics, chemistry, engineering—in short, all of the categories of western science. Never again, it is safe to assume, will such a question as "Why did China never develop a science?" be a proper one to ask.

The first volume of this seven-volume analysis of science in China is an introductory one divided essentially into five parts. The introduction lays down in detail the plan of the entire work; the problem of the language is then discussed in what is perhaps too minute a manner, for the non-sinologist will find it, at places, incomprehensible and the sinologist will find it unnecessary; this is followed by a short section (pp. 55-72) on the physical and economic geography of China which is most interesting and should prove of great value to the scientist and historian alike for the methods which are used in describing the relations of men to their geographical environment; then there is an historical introduction of some length (pp. 73-150) which attempts rather successfully to sketch in the history of China from the Stone Age to the seventeenth century; pages 150 to 248 deal with the enormous and utterly fascinating problem of cultural exchange between Europe and China in which Professor Needham sets down his well-documented ideas on the autonomy of Chinese culture while, at the same time, showing how this culture played a dynamic part in world civilization. Finally, the volume closes with an extraordinarily complete bibliography of Chinese and Western writings on Chinese (and Western) civilization.

To the historian of science, this first volume can be described only as tantalizing. Presented as he is with the tables of contents of the volumes to follow and being referred constantly in the text to these volumes for more detailed treatment of the scientific matters raised here, his reaction can be only one of anticipation. Professor Needham brings out in this volume most of the questions which he intends to answer later—what were the achievements of Chinese physics, chemistry, botany, metallurgy, medicine, engineering? What were the social, political, and economic conditions which prevented a "scientific revolution" from taking place in China? The answers, however, are not in volume one and we must be content to accept the framework here. That this framework, in general, is most skillfully erected cannot be denied. The historian of science cannot help but be impatient, however, for he is promised a feast by a man who has proven himself to be a first-rate scientist, an expert sinologist, and a top-notch historian. Let us soon have volume two!

L. PEARCE WILLIAMS

FORGOTTEN LEADERS IN MODERN MEDICINE; VALENTIN, GRUBY, REMAK, AUERBACH. By Bruno Kisch. Trans. Amer. Philos. Soc., New Series, vol. 44, part 2, pages 139-317. Philadelphia, The American Philosophical Society, 1954. $2.00.

These are the biographies of four little known, nineteenth century medical scientists. All were discoverers and contributed significantly to biology and medicine. Gabriel Gustav Valentin, for example, made the first observations on the enzyme diastase. Gruby, a busy physician, found time also for useful work in the fields of microscopy and mycology. Remak, a neurologist, not only discovered the nonmedullated nerve fibers with which his name is most often associated but showed that nerve fibers arise from

ganglion cells. Auerbach discovered the myenteric plexus and made fundamental observations on endothelium and on fertilization.

The accounts of the four lives are somewhat rambling but are related with warmth and sympathy. Professor Kisch's enthusiasm for his subjects occasionally results in eulogistic passages, but there can be no doubt that he has done well to remind us of how much these men achieved. The biographies are clearly the result of much careful research. Numerous excellent illustrations, thorough documentation, full bibliographies, and an index of names extend the usefulness and significance of this monograph.

THOMAS R. FORBES

FROM CLASSICAL TO MODERN CHEMISTRY. By A. J. Berry. New York, Cambridge University Press, 1954. 250 pp. $4.75.

In this volume Dr. Berry continues the study of the growth of chemical science begun in his *Modern Chemistry*. The volume is historical and traces the growth of today's knowledge from the beginnings made in previous centuries. The first chapter is introductory and gives a wide general view of the subject and the mental attitudes its pioneers have had to change or overcome. Subsequent chapters deal with single aspects of Heat; Electricity and Electrolysis; Physical Optics and Chemistry; Molecular Magnitudes; Analytical Chemistry; Chemical Formulae; Valency, Radicals and Constitution; Kinetic Energy. Very impressive in the light of today's techniques and equipment are the results and conclusions formed by 18th and early 19th century chemists. Not only did they work in the shadow cast by contemporary scientific giants, but their concept of mathematical analysis left them without adequate tools for a fuller evaluation of their findings. Despite these handicaps, men of all countries contributed, bit by bit, to the general, over-all advance of the science of chemistry. If an example were needed to point up the international character of science, then it is contained in this book.

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